MOLECULAR MODELLING, SYNTHESIS, CHARACTERIZATION & BIOLOGICAL EVALUATION OF SOME NOVEL 2-PHENYL 3-SUBSTITUTED AMINO QUINAZOLIN 4(3H)-ONES.

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University
Chennai-600 032

In partial fulfillment of the requirements
for the award of the degree of

MASTER OF PHARMACY

APRIL-2012

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
MADURAI - 625 020

Prof. (Mrs.) R. Tharabai, M.Pharm.,
Professor& Head of the Department,
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Madurai Medical College,
Madurai-20

CERTIFICATE

This is to certify that the dissertation entitled "***MOLECULAR MODELLING, SYNTHESIS, CHARACTERIZATION & BIOLOGICAL EVALUATION OF SOME NOVEL 2-PHENYL 3-SUBSTITUTED AMINO QUINAZOLIN 4(3H)-ONES*****"*** was done by **MISS. R. RIGANA MURSHIDHA, (Reg. No: 26108633)** in the department of pharmaceutical chemistry, College of Pharmacy, Madurai Medical College, Madurai-625020, in partial fulfillment of the requirement for the Degree of Master of pharmacy in pharmaceutical chemistry under my guidance and supervision for academic year 2011-2012.

This dissertation is forwarded to the Controller of Examination, The Tamil Nadu Dr. M. G. R. Medical University, Chennai.

Station: Madurai **Prof. (Mrs.) R. THARABAI, M.Pharm.,**
Date:

DR. (Mrs.) Ajithadas Aruna, M.Pharm, Ph. D.,
Principal ,
Head of the Department of Pharmacognosy,
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Station: Madurai **DR. (Mrs.) . Ajithadas Aruna,M.Pharm.Ph.D.,**

Date:

Evaluation Certificate

Internal Examiner

External Examiner

ACKNOWLEDGEMENT

I deem my great privilege to acknowledge all this eminent personalities who helped me throughout endeavor.

I extremely thankful to **Dr. A.EDWIN JOE, M.D.(F.M), Dean,** Madurai Medical College, for motivating us with constant encouragement and suggestions to complete this work successfully.

I cordially express my sincere thanks to **DR. (Mrs.). Ajithadas Aruna,M. Pharm., Ph. D.,** Principal & Head of the Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai for the support and encouragement of this project work.

It is my humble privilege to express to our heartfelt thanks to **Prof. (Mrs.) R. Tharabai, M.Pharm.,** Professor and Head of the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College , Madurai for giving this opportunity to work under her guidance, incessant encouragement, support in topic selection, supervision and completion of my project work in a successful manner.

I also express my heartfelt thanks to the tutors of department of pharmaceutical chemistry, College of Pharmacy Madurai Medical College, Madurai, Mrs. G. Umarani, M. Pharm., Mrs. G. Tamilarasi, M. Pharm., Mr. P. Sivasubramaniyan, M. Pharm.

I express my special thanks to DR. Chidambaram Rajan,M. Pharm., Ph.D., KM College of Pharmacy, Madurai, for his valuable support in completion of my project work.

I extend my thanks to Mrs.Indira, B.Sc., Mrs.Dharamambal, B.Sc., Mrs.Lalitha, B.Sc., lab supervisors, Department of Pharmaceutical Chemistry, Madurai Medical College.

I express thanks to Mrs. Radha, DMLT., Mrs. Sofiya, DMLT., lab technicians of Department of Pharmaceutical Chemistry, MMC, Madurai.

I also express special word of thanks to my juniors, Mr. T. Thirumalai Nambi, Mr. K. Yuvarajan, Mr. R.Jegadesh, Miss. J.Soniya in the department of pharmaceutical chemistry, College of Pharmacy, Madurai Medical College for their fruitful discussion and endless help to complete this work successfully.

I express my special thanks to Mr. Jones Kumar for supplying the necessary chemicals.

I express my special thanks to Mr. Sivakumar, M. Pharm., RVS College of Pharmacy, endless help to complete this work successfully.

(For IAE / CPCSEA usage)

Proposal number	: RRM/R.Rigana murshidha/ 26108633/M.Pharm/MMC/ 2010-2012/MGRMU
Date first received	: 15/11/2011
Date received after modification (if any)	: Nil
Date received after second modification (if any)	: Nil
Approval date	: 22/11/2011
Expiry date	: 30/11/2012
Name of IAEC / CPCSEA chairperson	: N.CHIDAMBARANATHAN

Date:22.11.11

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CONTENTS

LIST OF ABBREVATIONS

^0C	:	Degree Centigrade
%	:	Percentage
gm	:	Gram
mg	:	Milligram
Kg	:	Kilogram
ml	:	Milliliter
IR	:	Infra Red
mol	:	Mole
QSAR	:	Quantitative Structural Activity Relationship
Ar	:	Aromatic
R_f	:	Retention factor
Str.	:	Stretching
Ph	:	Phenyl
5-FU	:	5-flurouracil
DAL	:	Dalton Ascites Lymphoma
DMSO	:	Dimethyl sulfoxide
mm	:	millimeter
M.Wt	:	Molecular weight
M.f	:	Molecular formula
W/V	:	weight per volume

Chapter-I

INTRODUCTION

Medicinal Chemistry is a science whose roots lie in all branches of chemistry and biology. Pharmaceutical Chemistry is a branch of Science that makes use of the general law of chemistry to study drugs (ie) their preparation, chemical nature, composition, studies of their physical and chemical proportion, method of quality control and the conditions of their usages.

The earlier sources of drugs were from plants, animals and mineral sources, but due to the lack of potential action and definitive cure and sometime more toxicity, the discovery of new drugs that are more potential and less toxic is essential. The synthesis of derivatives has been an important part and is aimed at modifying the action of drugs, particularly to reduce the side effects and to potentiate the drug action. Today more than 60% drugs used in practice are synthesized derivatives and day-by-day the scope of synthetic medicinal chemistry is broadening.

Once a new pharmaceutical lead compound has been identified, extensive and costly effects usually are made to prepare a series of analogue in the hope that even better activity will be found.

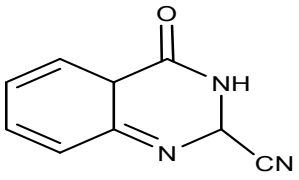
Medicinal chemistry is a highly interdisciplinary science combining organic chemistry with biochemistry, computational chemistry, molecular biology, genetic engineering, pharmacology, statistics and physical chemistry. Medicinal chemists have a chance to participate in the fundamentals of prevention therapy and understanding of diseases by providing the drugs either through discovery (or) through design.

It remains a challenging science which provides profound satisfaction to its practitioners.

QUINAZOLINES – AN OUTLOOK

QUINAZOLINES[2]

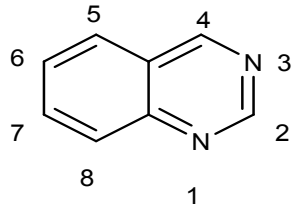
In 1869 Griess prepared the first quinazoline derivative, 2 – cyano 3, 4-dihydro-4-oxoquinazoline, by the reaction of cyanogens with anthranilic acid. Griess apparently recognized the bicylic nature of the product which, he called bicyanoamido benzoyl and used this name until 1885. When structure (1) was known with some certainly.



(1)

The preparation of the parent quinazoline was discovered after years, later when Bischler and Lang obtained it by de-carboxylation of the 2-carboxy derivative.

A more satisfactory synthesis of quinazoline was subsequently devised by Gabriel in 1903 that studied properties and those of its derivatives in greater detail.



The name was proposed by Widdege. Other names such as phenmiazine, benzyleneamidine, benzo-1,3-diazine,5,6-benzopyrimidine and 1,3-diazanapthaline

have occasionally been used. The numbering suggested by Paal and Busch is still in use.

The presence of a fused benzene ring alters the properties of the pyrimidine ring considerably. The two nitrogen atoms are not equivalent, and the marked polarization of the 3, 4- double bond is reflected in the reactions of quinazoline. The properties of substituted quinazolines depend largely on,

a. The nature of the substitutents.

b. Whether they are in the pyrimidine ring (or) in the benzene ring

c. Whether (or) not complete conjugation is present in the pyrimidine ring.

The chemistry of quinazoline was reviewed by Williamson in 1957, then by Lindquist in 1959 and brought up to date by Armarego in 1963.

CHEMICAL PROPERTIES

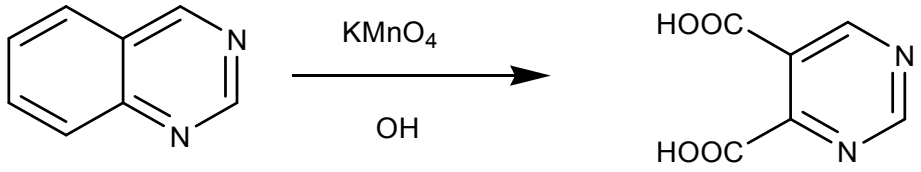
Quinazolines is stable in cold dilute acid and alkaline solutions, but it is destroyed when these solutions are boiled. When quinazoline is boiled with HCl it forms *o*-aminobenaldehyde, amine and formic acid.

a) Hydrolysis, oxidation and reduction.

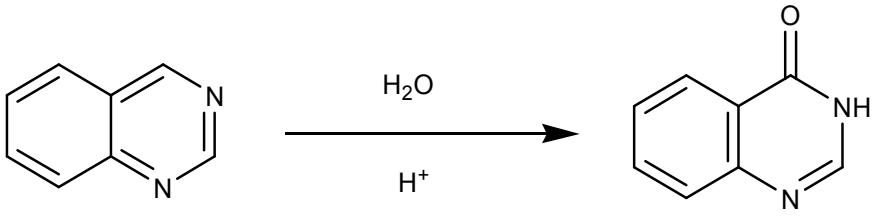
Oxidation of quinazoline in dilute aqueous acid, with two equivalents of hydrogen peroxide at room temperature resulted in the formation of a high yield of 3, 4- dihydro-4-oxo quinazoline.

In alkaline medium, where the anhydrous neutral species of quinazoline were predominantly undergo oxidation with $KMnO_4$ furnished a high yield of 3, 4-dihydro-4-oxo quinazoline was also formed.

Oxidation



Reduction



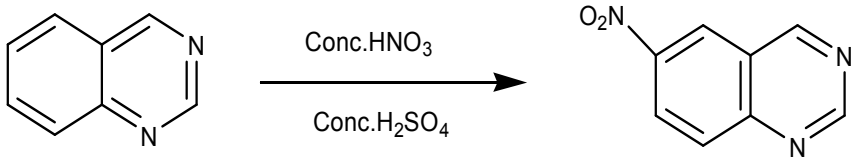
Catalytic hydrogenation of quinazoline stopped after the absorption of one molecules of hydrogen and gave 3,4-dihydro quinazoline.

Reduction with sodium amalgam gave 1,2,3,4-tetrahydroquinazoline. Lithium aluminum hydride and sodium borohydride gave 3,4-dihydro and 1,2,3,4-tetrahydroquinazoline.

b) Nucleophilic and electrophilic substitution reactions

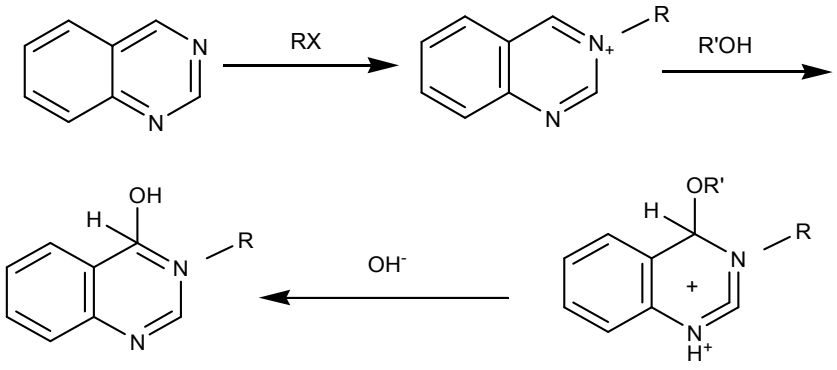
The two known nucleophilic substitution reactions of quinazoline namely with sodamide and hydrazine, presumably proceed via the intermediate addition products and gave 4-amino and 4-hydrazine quinazoline.

Nitration is the only known electrophilic substitution reaction of quinazoline. Theoretical considerations show that the expected order of reactivity is at positions 8 > 6 > 5 > 7 > 4 > 2. Quinazoline gives 6-nitroquinazoline with fuming nitric acid in concentrated H_2SO_4. No oxidation of the heterocyclic ring can occur under these conditions because the hydrated cation is not present.



c). Alkylation reactions

Alkylation of quinazoline takes place on N,3-methyl,3-ethyl-3-alkyl and 3-benzyl quinazolinium salts readily take up a molecule of alcohol to form the corresponding 4-alkoxy-3-alkyl-3,4-dihydro quinazolinium salts. These salts yield the pseudo bases, 3-alkyl-3, 4-dihydro-4-hydroxy quinazolines on treatment with strong alkali.



d). Addition reactions

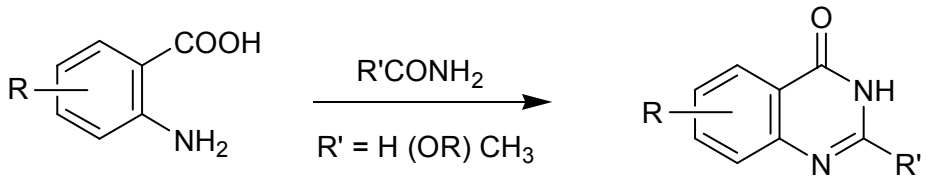
Quinazoline is very reactive towards anionic reagents which attack position 4. Sodium bisulphate, hydrogen cyanide, acetophenone, acetone, 2-butanone and cyclohexanone add across the 3,4-double bond of quinazoline. Methyl, ethyl, isopropyl, benzyl, t-butyl and phenyl magnesium halides and phenyl lithium also add across the 3, 4-double bond to give the corresponding 4-substituted 3, 4-dihydroquinazolines.

SYHTHESIS

Following methods were reported for the synthesis of oxoquinazolines.

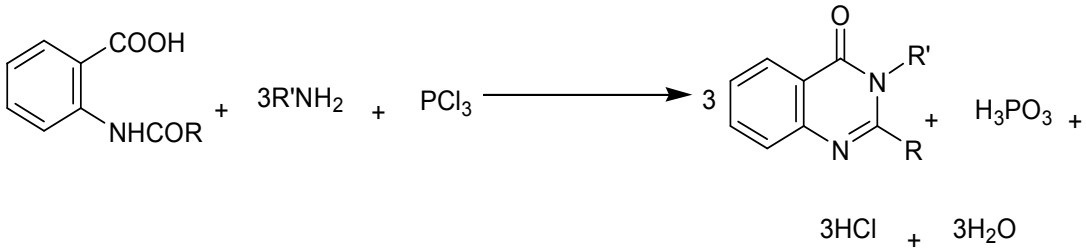
a) Niementowski's synthesis

Niementowski's found that 3 (or) 4 substituted anthranilic acid when reacted with formamide at 125 - 130ºC for 4 hours gave 86% yield of 3, 4-dihydro-4-oxoquinazoline.



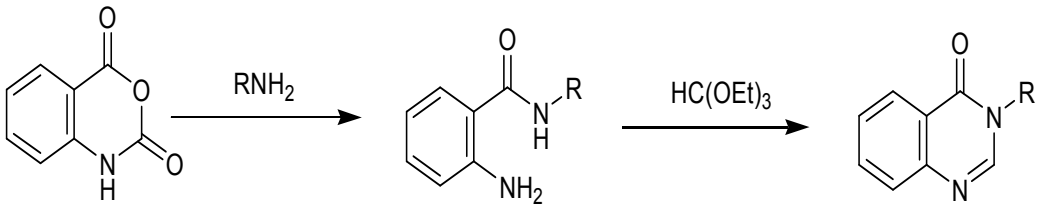
b) Grimmel, Guinther and Morgan's synthesis.

3 moles of *o*-amino benzoic acids, when heated with 3 moles of an amine together with one mole of phosphorous trichloride in toluene for two hours, gave high yields of 2,3-disubstituted 3,4-dihydro-4-oxoquinazolines.



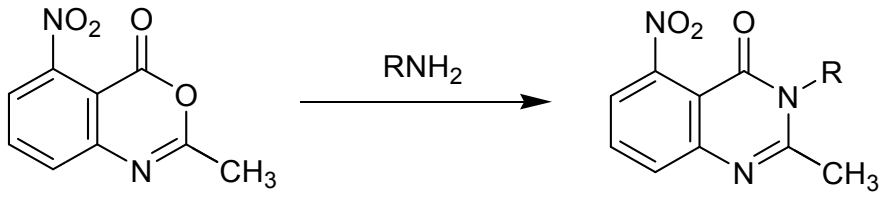
c) From isatoic anhydride

Isatoic anhydride readily reacts with equimolar quantity of amines to dihydro-4-oxoquinazolines by refluxing ethyl orthoformate for 1- 6 hours without isolating the intermediate amides.



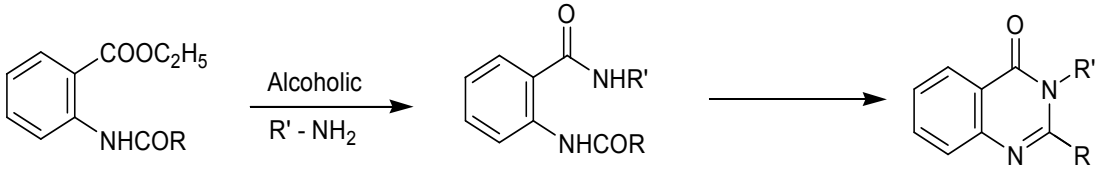
d) From1,3,4-Benoxazones (Acylanthranils) and amines.

1,3,4-Benoxazones react with amines to give 3,4-dihydro-4-oxoquinazolines. Primary aliphatic amines and anilines react with 2-methyl-5-nitro-4-oxoquinazolines.



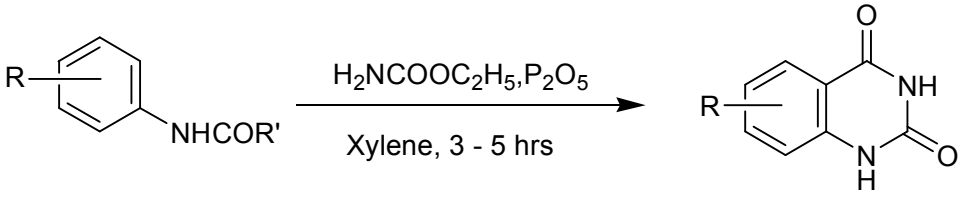
e) From ethyl 2-acetamido-5-nitrobenzoate.

Ethyl 2-acetamido-5-nitrobenzene and alcoholic ammonia when heated in a sealed tube at 170ºC, yields 3,4-dihydro –methyl-6-nitro-4-oxoquinazoline.



f) Sen and Ray's synthesis

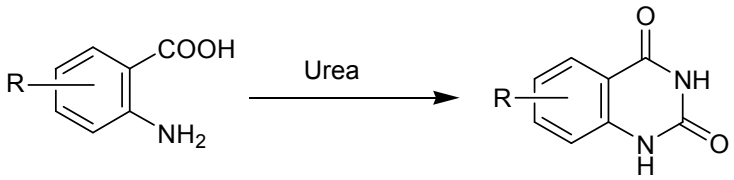
Boiling a solution of normal (or) isobutyrylanilides with urethane and phosphorous pentoxide in the presence of xylene gave 2-propyl and 2-isopropyl-3, 4-dihydro-4-oxoquinazolines.



(R = Me, OMe, OEt; R' = Me, Et, Pr, Iso-Pro, Ph)

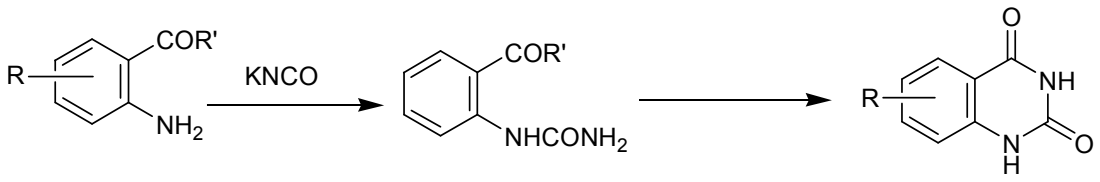
g) From anthranilic acid and ureas.

The fusion of anthranilic acid with urea to give 1,2,3,4-tetrahydro-2,4-dioxoquinazoline was first described by Griess.



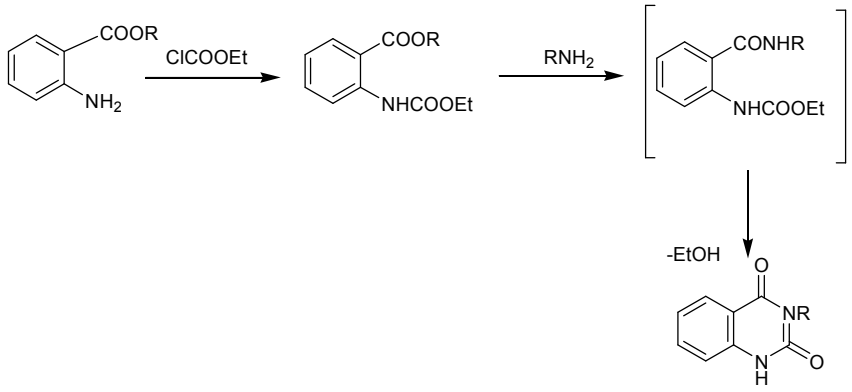
h) From *o*-ureidobenzoic acid.

o-ureidobenzoic acids are readily prepared from the corresponding anthranilic acid and potassium cyanate. The ureido acids are then easily cyclised to the respective 1,2,3,4-tetrahydro-2,4-dioxoquinazolines by heating with acid (or) alkali. Anthranilic esters and amides as well as undergo this reaction.



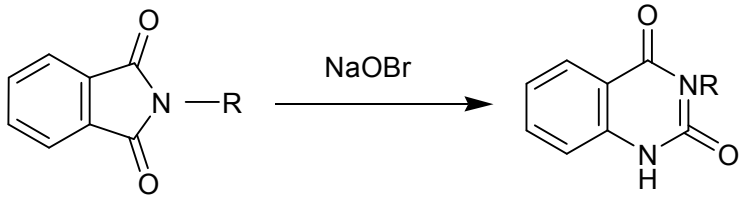
i) From *o*-ethoxy carbonylaminobenzoic esters (or) amides.

When *o*-ethoxy carbonylamino benzamide and its 4-methyl derivatives are heated above their melting points, they lose water and from 1,2,3,4-tetrahydro-2,4-dioxoquinazoline.



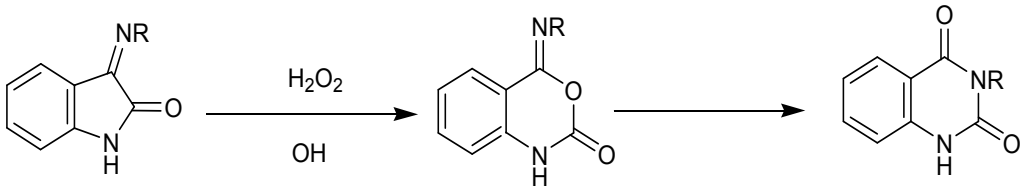
j) From phthalic acid derivatives.

The use of derivatives of phthalic acid for the preparation of dioxoquinazoline necessitates rearrangement of the Hoffmann cruties (or) Lossan type. Reaction of phthalamide (or) phthalimide, N-methyl and N-ethyl phthalimide with alkali hypobromite gives the 1,2,3,4-tetrehydro 2,4-dioxoquinazoline.

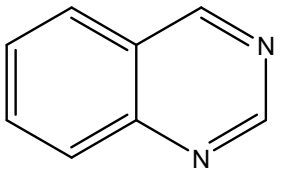


k) From Isatins

α-isatin oxime rearranges to 1,2,3,4-tetrahydro-2,4-dioxoquinazoline on heating with dilute sodium hydroxide, β-imino derivatives of isatin, on the other hand, require oxidation with hydrogen peroxide in alkaline solution in order to from the dioxoquinazoline.



Quinazoline is an aromatic benzopyrimidine ring system. It was earlier known as benzo 1,3-diazine, first prepared by Gabriel, in 1903.

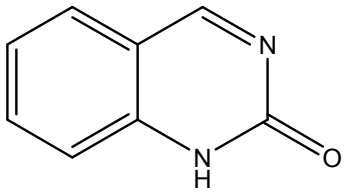


Quinazoline

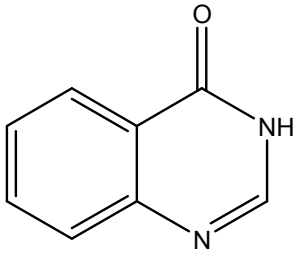
It was isomeric with the compounds, cinnoline and quinoxaline of the many derivatives of quinazoline system known so far, keto quinazolines also called as quinazolinones are the most important compounds.

Depending upon the position of the Keto (or) Oxo group, these compounds may be classified into two types,

1. 2-(1H) Quinazolinone
2. 4-(3H) Quinazolinone



Quinazolin -2(1H)-one



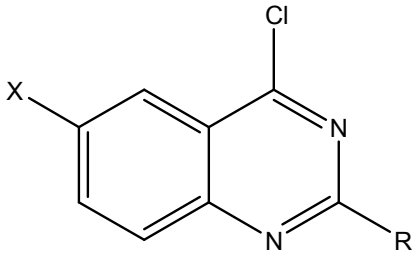
Quinazolin -4(3H)-one

The structure activity relationship studies of Quinazolinone ring system revealed in various literature suggests position 2,6,8 are very much important for structure activity studies and position 3 should be attached to different heterocyclic rings for better chemotherapeutic activity.

From the reviews of various literatures, it was known that Quinazolin-4-ones have emerged as an important class of nitrogenated heterocycles that have attracted significance synthetic interest because of their therapeutic and pharmacological properties such as Antibacterial, Antifungal, Anthelmintic, Anti-inflammatory, Anticonvulsant, CNS depressant, Hypoglycemic, Antiparkinsonian, Anticancer, Antiviral, Antihistaminic, Antihypertensive, Analgesic and many other activities.

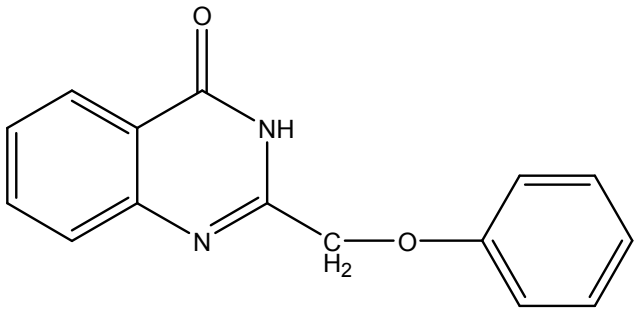
Literature Review

1. P. Mani Chandrika et al, synthesized novel 2,4,6-tri- Substituted Quinazoline derivatives Antibacterial and cytotoxic agents.

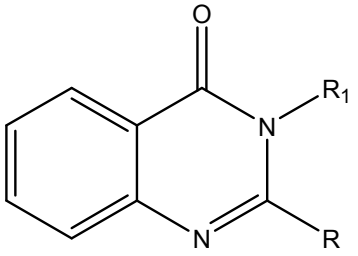


X = H
R = C_6H_5, CH_3, CF_3, I, Br

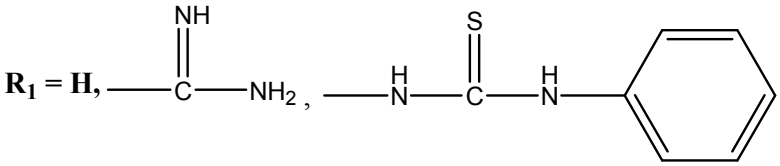
2. L. Cipak et al, synthesized 2-phenoxy methyl 3-H-Quinazolin-4-one as Anticancer agents.



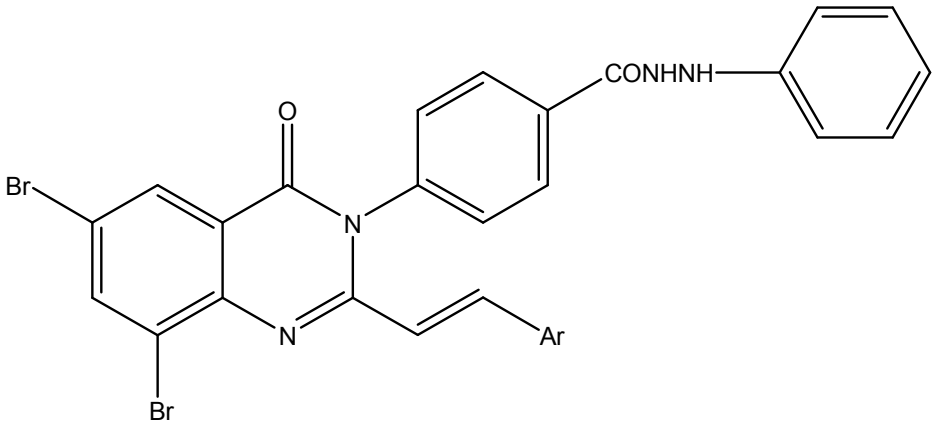
3. R.K. Kawadkar, B.J. Ghiya reported on synthetis of new Quinazolin-4-one compounds of medicinal importance.



R = CH_3, C_6H_5

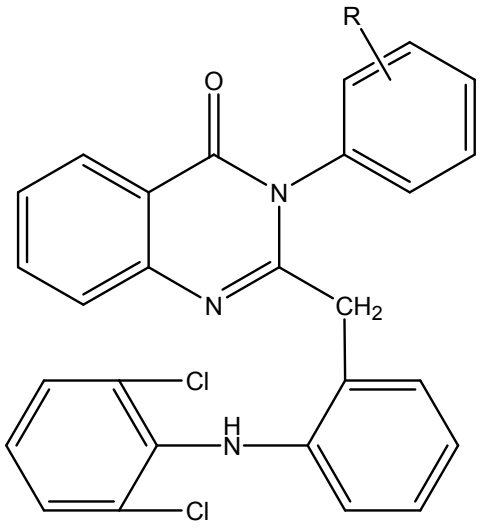


4. V. Murugan et al, reported on the synthesis of 2-substituted Quinazolin-4(3H)-ones as a new class of Anticancer agents.



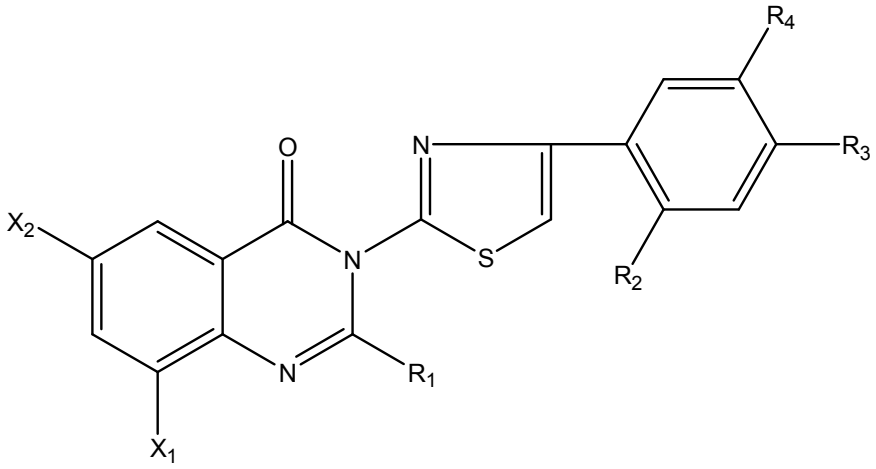
Ar = -C_6H_5, -CH=CH-C_6H_5, -M-C_6H_5-NO_2

5. N.B. Patel and J.D. Lilakar reported on the synthesis and Antibacterial activity of new substituted 4-(3H)-Quinazolinones.



R = H, 2-NO_2, 2-CH_3, 2-OOH_3, 2-Cl, 3-NO_2, 3-CH_3, 3-OCH_3, 3-Cl

6. P.C. Sarkar et al, reported on the synthesis and biological evaluation of some new 2-aryl/substituted aryl 6,8-substituted Quinazol-4(3H)-ones.

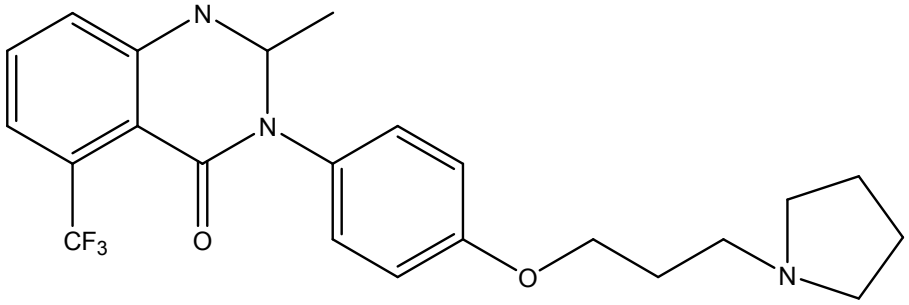


$X_1 = X_2 = R_2 = R_3 = R_4 = H$ **$R_1 = C_6H_5$-**

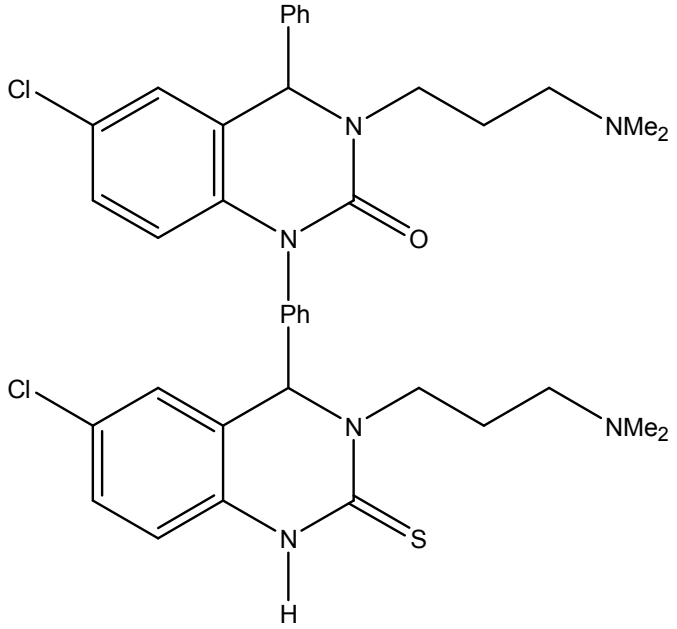
$X_1 = X_2 = R_2 = R_3 = R_4 = H$ **R_1 = P-Cl C_6H_5-**

$X_1 = X_2 = R_2 = R_3 = R_4 = H$ **R_1 = M-Cl C_6H_4-**

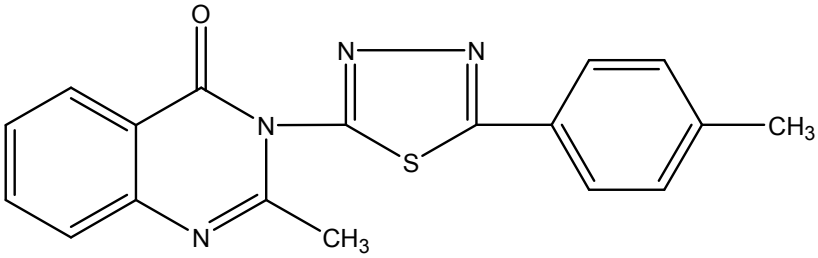
7. Tsuyoshi Nagase, Takashi Mizutani et al, synthesis and evaluation of structurally constrained Quinazolinone derivatives as potent and selective histamine H3 receptor inverse agonists.



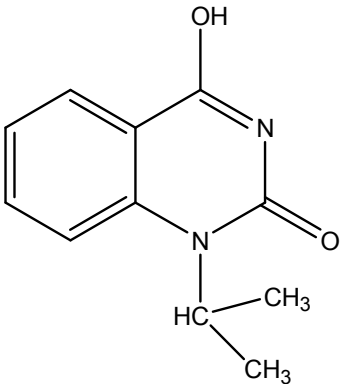
8. Hirohiko Hasegawa, Masami Muraoka et al, discovery of a novel potent Na^{+}/Ca^{+2} exchanger inhibitor. Design, synthesis and structure activity relationships of 3,4-Dihydro-2(1H)-Quinazolinone derivatives.



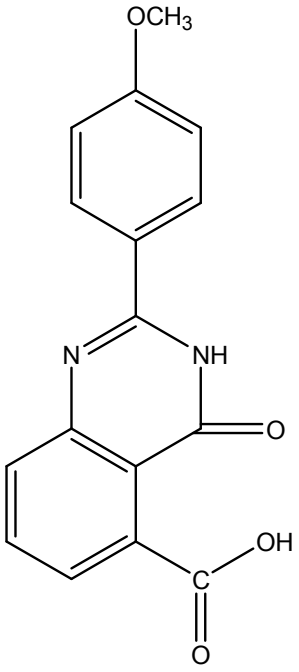
9. Jatav Varsha et al, reported on the synthesis and Antimicrobial activity of novel 2-methyl-3(1,3,4-Thiadiazoyl) 4-(3H) Quinazolinone.



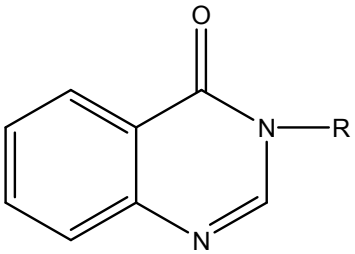
10. Perrine JW et al, reported on the Anti-inflammatory and other pharmacodynamic properties of five members of the 4-aryl-1-isopropyl-2(1H)-Quinazolinones.



11. Lyman R. Caswell et al, reported on the synthesis of 2-aryl-4(3H)-Quinazolinone-5-carboxylic acids.

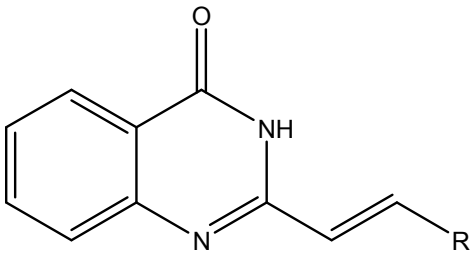


12. Mazaahir Kidwai et al, reported on the novel route to the Neimentowski reaction for Quinazolinone synthesis.



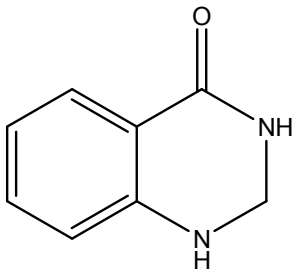
R = 3-Chloro, 4-Fluorophenyl, 2-fufuryl, 2-pyridyl

13. I. Philipova et al, reported on the synthesis of some 2-Substituted 4-(3H)-Quinazolinone derivatives.

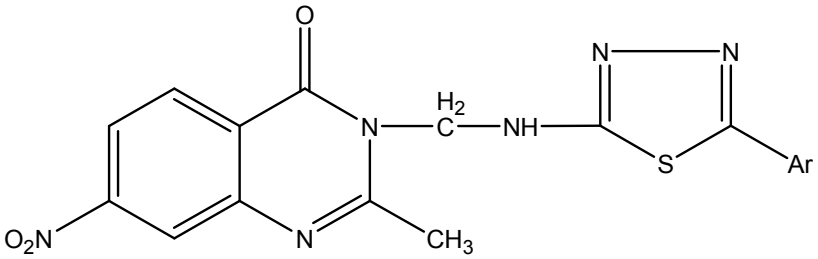


R = aromatic aldehydes

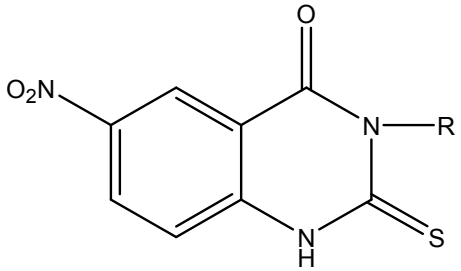
14. N.A. Gangwal et al, reported on the synthesis and QSAR studies of substituted 4(1H)-Quinazolinones.



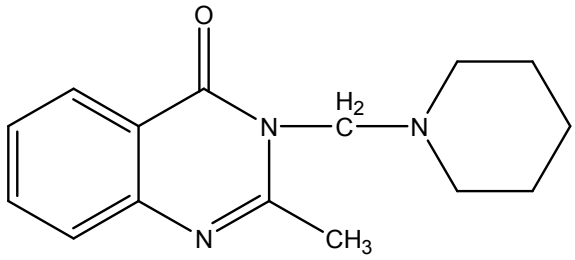
15. A.R. Bhat et al, reported on synthesis and biological activities of Mannich bases of 7-nitro 2-methyl 4(3H)-Quinazolinones.



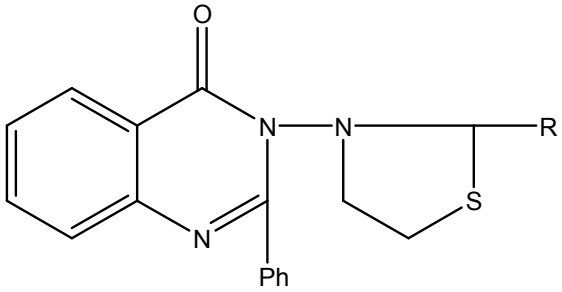
16. Ram Lakhan and Rakesh K. Banerjee et al, reported on a simple preparation of 2-thioxo-4(3H) Quinazolinones.



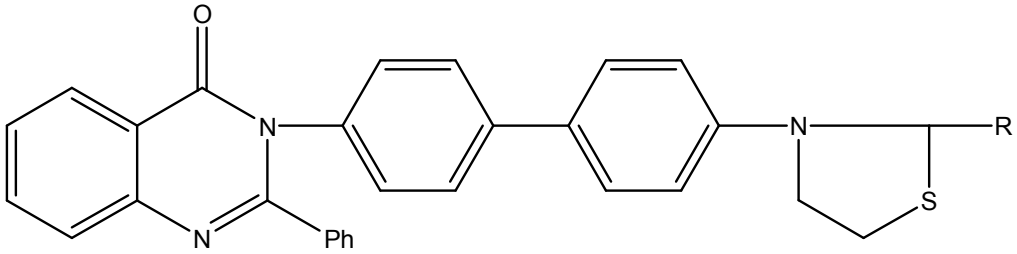
17. M.A. Aziza et al, reported on the synthesis and Antimicrobial activities of somenew 3-heteroaryl Quinazolin 4-ones.



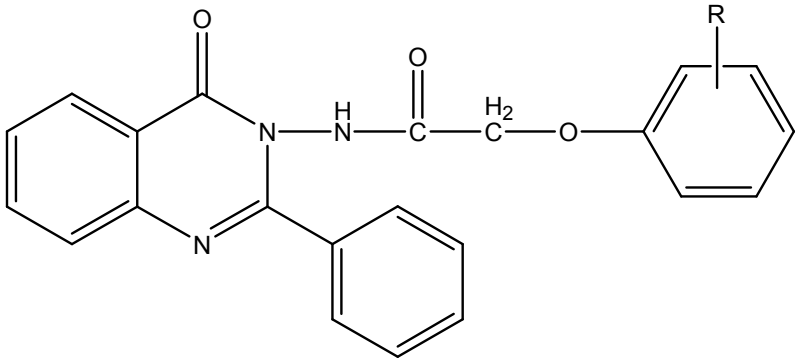
18. Krishna Srivastava et al, reported on the synthesis of thiazolyl Quinazolines for studying their Antiviral activity against Japanese encephalitis virus, a RNA virus of high pathogenicity.



R = Different aromatic aldehydes



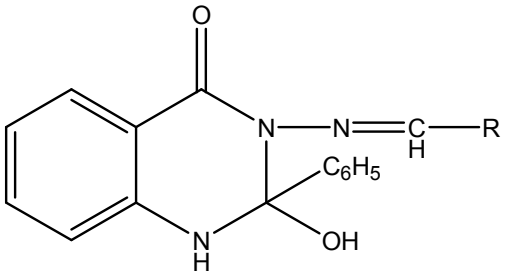
19. Deepti Kohli et al, reported on the synthesis and Antibacterial activity of Quinazoline derivatives.



R = Substituted Phenols

20. YA Ammar, YA Mohamed et al, reported on the synthesis of some biologically active 4(3H) Quinazolinones derived from 2,3-pyridine dicarboxylic anhydride.

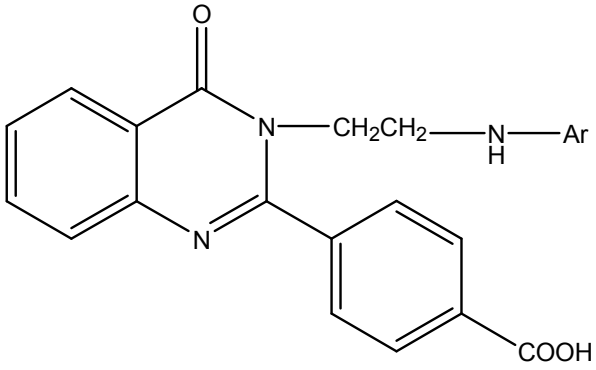
21. Pankaj S. Salunkhe et al, reported on the study of analgesic and Anti-inflammatory evaluation of some 2,3-Dihydro Quinazoline 4-one derivatives.



R = Different aldehydes

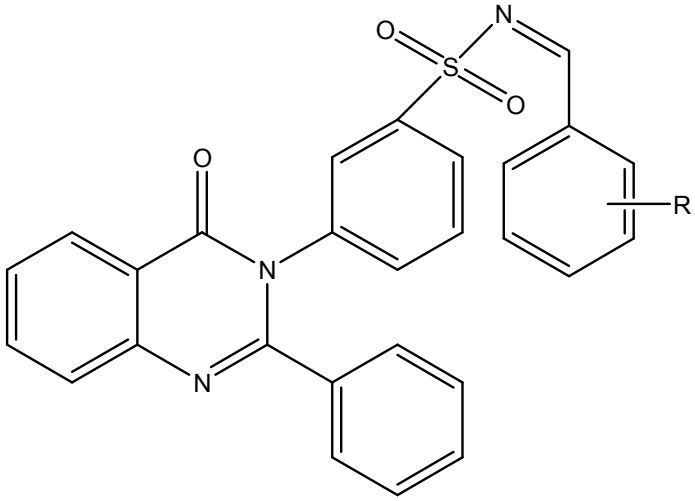
22. Mosaad S. Mohamed and Mohsen M. Kamel et al, reported on the synthesis, biological evaluation, and Molecular docking of Quinazoline-4(1H)-one derivatives as Anti-inflammatory and analgesic agents.

23. B. Kiruthiga, K. Ilango et al, reported on the synthesis of some new 2-substituted Quinazolin-4-one derivatives and their biological activities.



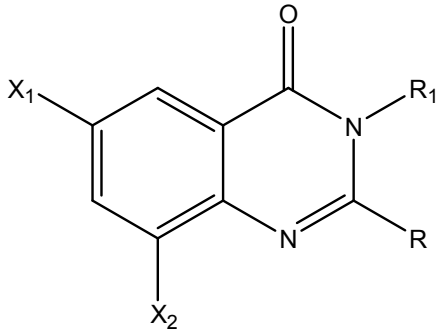
Ar = Aromatic Amines

24. Dhansay Dewangan and D.K. Tripathi et al, reported on the synthesis, characterization and Anti-inflammatory Analgesic and Antimicrobial activities of substituted 4-(4-oxo-2-phenyl Quinazolin-3(4H)-yl) N-aryl-methylene benzene sulfonamide derivatives.



R = Aromatic aldehydes

25. K. Hemalatha and K. Girija et al, reported on the synthesis of some novel 2,3-disubstituted Quinazolinone derivatives as analgesic and Anti-inflammatory agents.

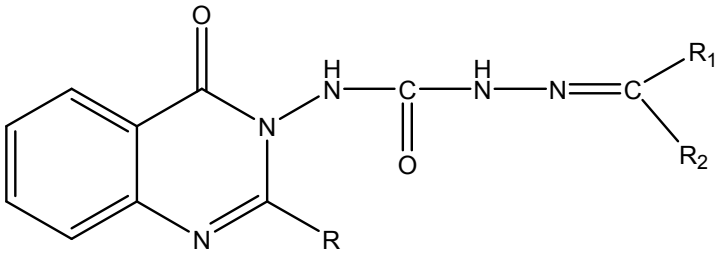


X1, X2 = H, Br

R = -CH_3, -C_6H_5

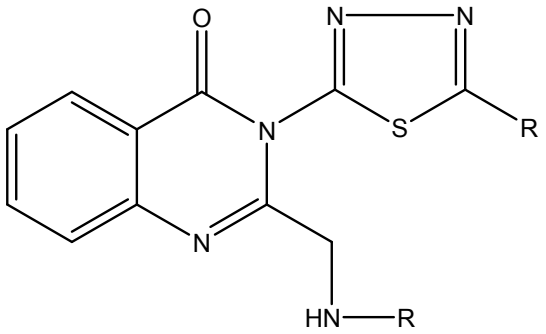
R_1 = -$C_6H_4CH_3$, -$NHCOC_6H_4N$

26. Ponnilavarasan Ilangovan and Swastika Ganguly et al, reported on the design and synthesis of novel Quinazolinone derivatives as broad spectrum Anticonvulsant and Antimicrobial agent.



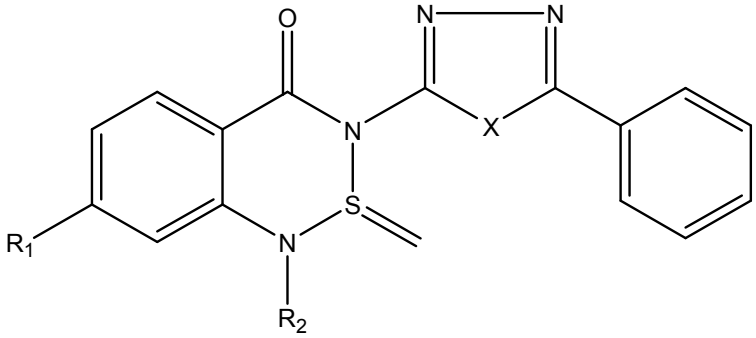
R = Phenyl
R_1 = H
R_2 = 2-OH-Phenyl, 4-OH-Phenyl
R_1 = R_2 = Furan,Isatin, Phenyl

27. Jayshari S. Pattan et al, reported on the synthesis and evaluation of some new Quinazolone derivatives for their Antimicrobial activity.



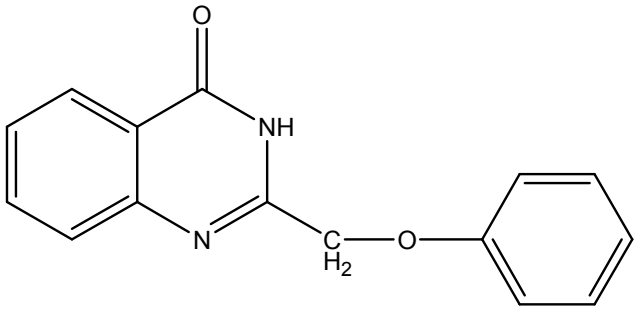
R = Different Amines

28. Vinod K. Tiwari et al, synthesized 3-heteroaryl-2- thioxo-2,3-dihydro Quinazolin-4-(1H)-one.

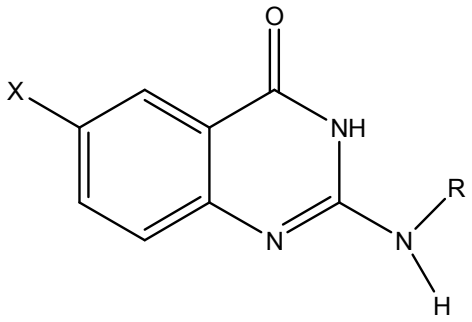


X = O, S
R = H, Cl
R_1 = H, CH_3
R_2 = CH_3, H

29. L. Cipak et al, synthesized 2-phenoxy methyl 3-H Quinazolin-4-one as Anticancer agents.

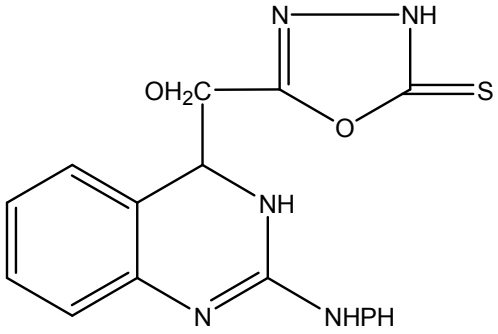


30. Bernard Pirotte et al, reported on the synthesis and K_{ATP} channel activity of 2-alkyl amino 6-halogeno Quinazolin-4(3H)-ones.

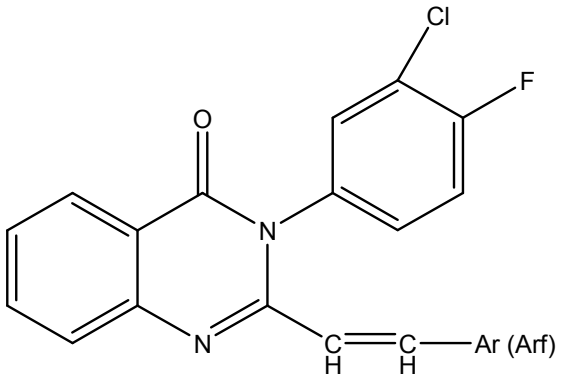


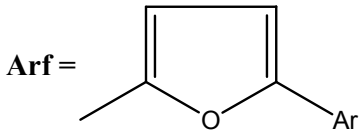
X = Cl, I R = $CH(CH_3)_2$
X = Cl, I R = $CH(CH_3)\,CH_2CH_3$
X = Cl, I R = $CH(CH_3)\,CH(CH_3)_2$
X = Cl, I R = $CH_2C_6H_{11}$

31. M.E. Abd El-Fattah reported on the synthesis of 4-substituted 2-phenyl amino Quinazolines.

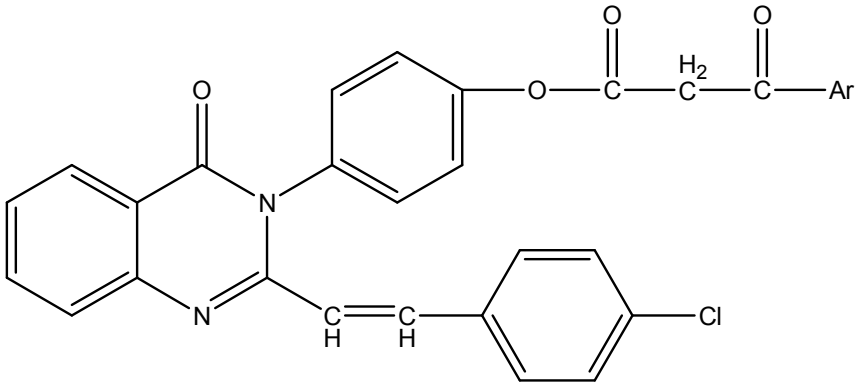


32. B. Shivarama Holla, SHalini Shenoy et al, reported on the synthesis and Antibacterial activity of some fluorine containing aryl furyl vinyl Quinazolinones.

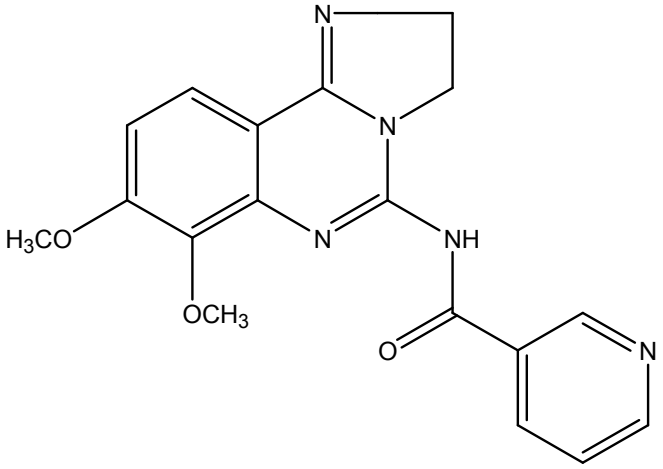




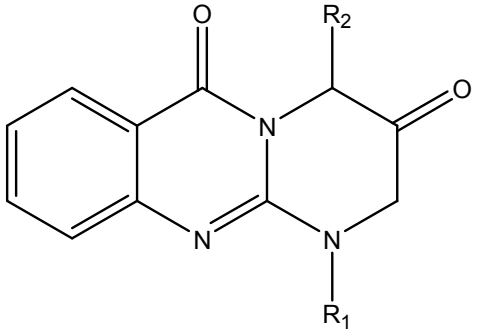
33. S. El-Meligie, AK El-Ansary et al, reported on the synthesis and Antimicrobial activity of 2-(2-aryl vinyl)-7-substituted-Quinazolin-4(3H)-ones.



34. Zachary A Knight et al, reported on the pharmacological map of the P13-K family defines a role for P110X in insulin signaling.

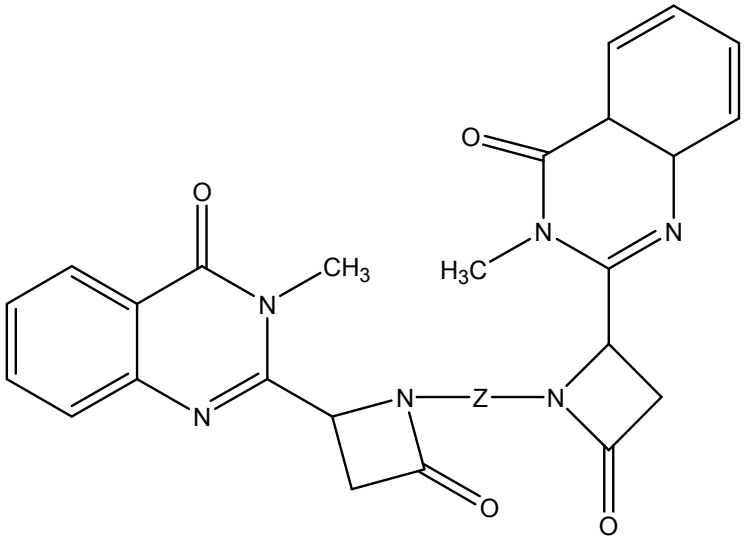


35. Chang Xie et al, synthesized imidazo [2,1-b] Quinazoline-2,5(1H,3H)-diones.

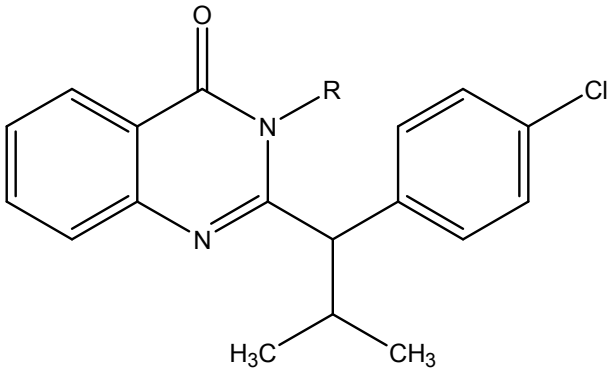


R_1 = Ph, 4-Chlorophenyl, 3-Methyl phenyl
R_2 = H, CH_3, $C_6H_5CH_2$

36. PSN Reddy et al, reported on the synthesis of novel bis Quinazolinonyl-β-lactams.

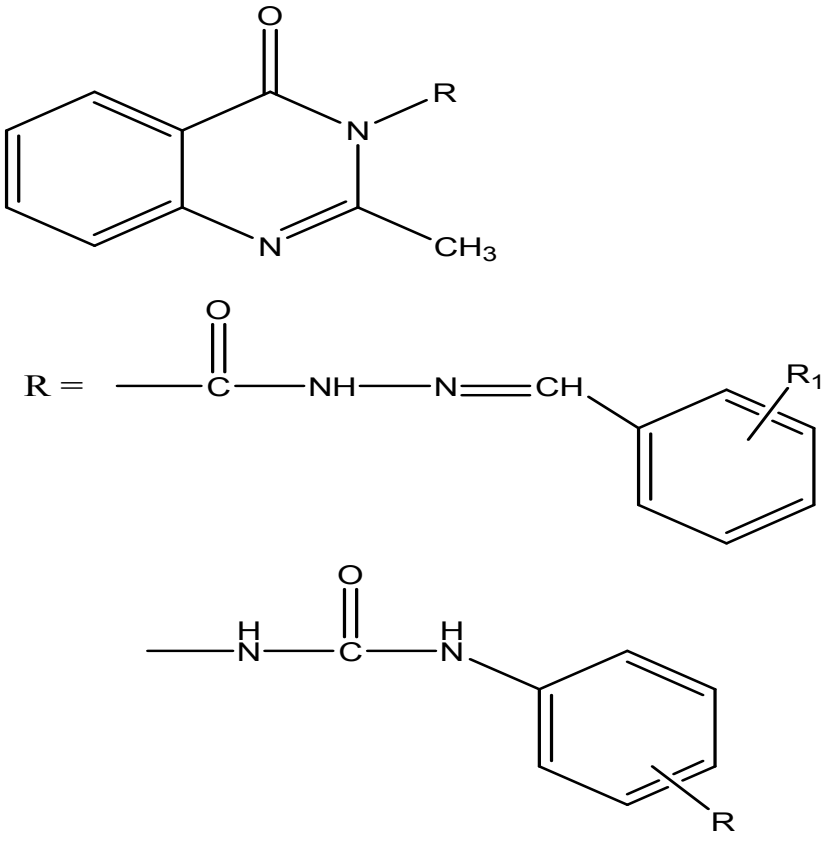


37. D.M. Purohit, V.N Patolia reported on the synthesis and Antimicrobial activity of 2-(1'[4-chlorophenyl)-2'-methyl propyl]-3N- (aryl) Quinazolin-4(3H) ones.



R = Aryl

38. NIzamuddin, Manoj Kr Srivastava reported on the synthesis, Antibacterial, Antiviral activity of some 2-methyl-3-(aryl thio-carbamido) Quinazol-4-ones and 2-methyl-3-cayliden-carboxomido) Quinazol-4-ones.



39. Quinazolinones as Anti-inflammatory agents: m. Jonathan Fray et al, reported on the synthesis of substituted 2-amino 4-Quinazolines via ortho fluoro benzoyl guanidines as Anti-inflammatory agents.

40. Emilan Geo Gescu, Floretina Georgescu, Mino R. Caira Alina Micolescu, Calin Danila, Petru Fillip and Florea Dunutrascu, A new synthesis of pyrrolo (1,2-C) Quinazoline from Quinazolinium n-ylides a re-investigation, ARKIVOC (2009), 232-241.

41. Deepti Kohli, S. Riaz Hashini, S. Vishal, Manish Sharma and Ashutosh Kumar Singh, synthesis and Antibacterial activity of Quinazolinone derivatives, International Journal of Pharmacy and Pharmaceutical Sciences, (2009), 1, 163-169.

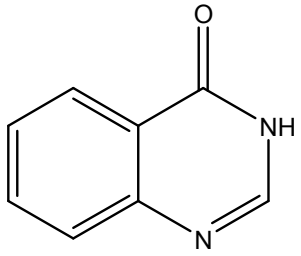
42. Juhhui You, Changwen Ye, Yabriao Weng, Xihao Mo and Yuliang Wang, synthesis and Anticoccidial activity of 4-(2-methoxy phenyl)-2-oxobutyl Quinazoline derivatives, ARKIVOC (2008), (XVII) 1-11.

Chapter-II

Chapter II

Objective of present work

Quinazolin-4-(3H)-one is a versatile lead molecule for the design of potential bio-active agents.



Quinazolin -4(3H)-one

From the literature review, it was known that most of the Quinazolin-4(3H)-ones having substitution at C-2 and N-3 positions possess various interesting pharmacological activities.

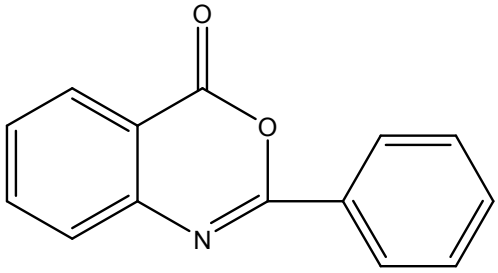
In the present work, Novel 2-phenyl-3-amino (substituted) quinazolin-4(3H)-ones were synthesized and their Anticancer and Anti-inflammatory activities were evaluated.

The objective of the present work can be summarized as follows:

I) Synthesis:

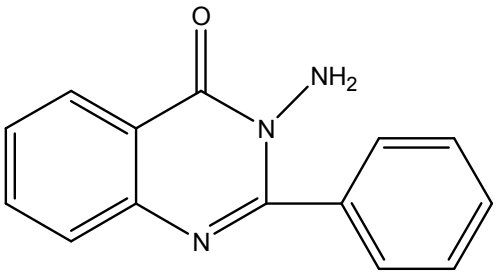
To synthesize 2-phenyl-3-substituted amino quinazolin-4(3H)-one derivatives by the following steps

- First Step is the cyclization of Anthranilic acid with benzoyl chloride to give 2-phenyl-4H-3,1-benzoxazine-4-one by Schotten-Bauman reaction.



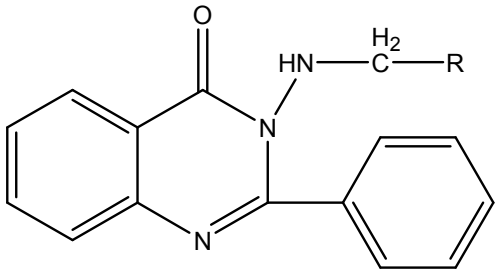
2-Phenyl-4H-3,1-benzoxazin-4-one

- Second step is the condensation of 2-phenyl-4H-3,1-benzoxazine-4-one with hydrazine hydrate to yield 3-amino-2-phenyl quinazolin-4(3H)-one.



3-Amino-2-phenyl quinazolin -4-(3H)-one

- Third step is the substitution of various amines to yield 3-substituted amino-2-phenyl- quinazolin-4(3H)-one.



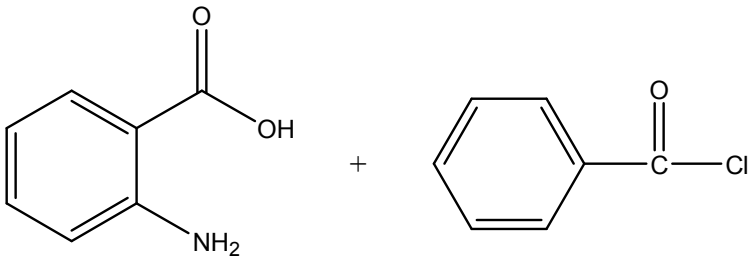
II) Characterization:

Characterization of the synthesized compounds by the analytical techniques like melting point, Thin layer chromatography, Infrared spectral analysis, Nuclear magnetic resonance spectral analysis, mass spectral analysis methods.

III) Pharmacological screening

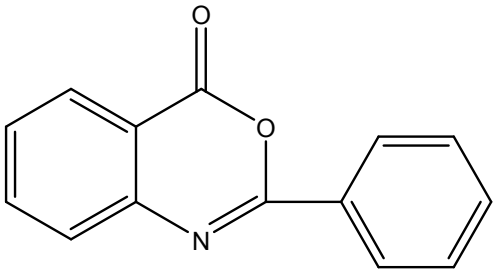
- Screening of the synthesized compounds for Anticancer activity using Dalton's lymphoma ascities cells in Swiss albino mice.
- Screening of the synthesized compounds for Anti-inflammatory activity using the carrageenan rat paw edema model.

Scheme of reaction



Anthranilic acid **Benzoyl Chloride**

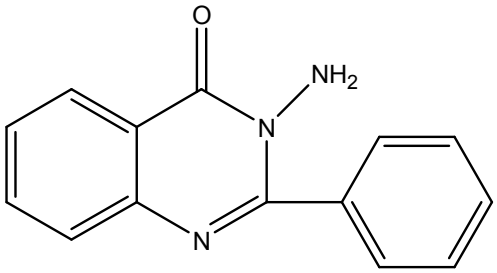
Pyridine



2-Phenyl-3,4-dihydro benzoxazin-4-one

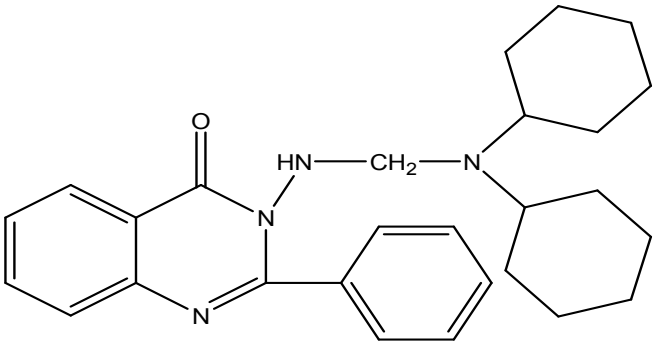
NH_2 - NH_2 . H_2O

Ethanol



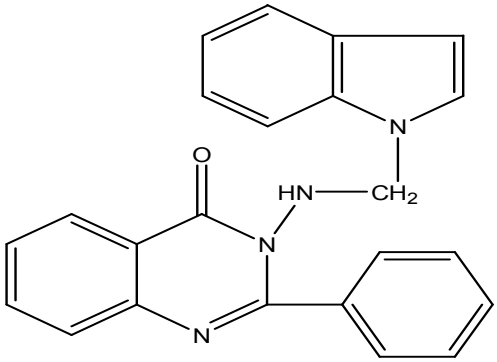
3-amino-2-phenyl quinazolin-4(3H)-one

S1



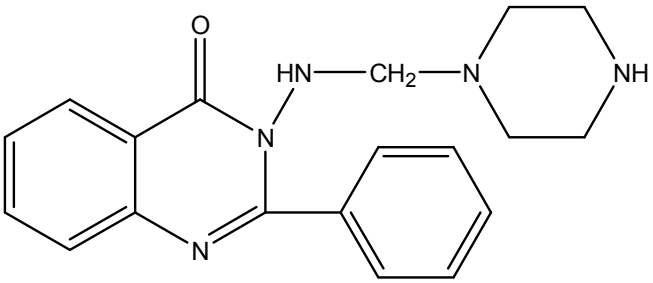
2-phenyl-3{[(dicyclohenyl amino) methyl] amino}- quinazolin-4(3H)-one

S2



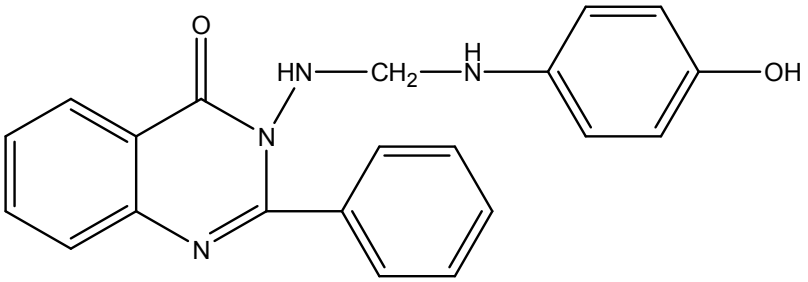
2-phenyl-3{[(1H-indol-1-yl methyl) amino]}- quinazolin-4(3H)-one

S3



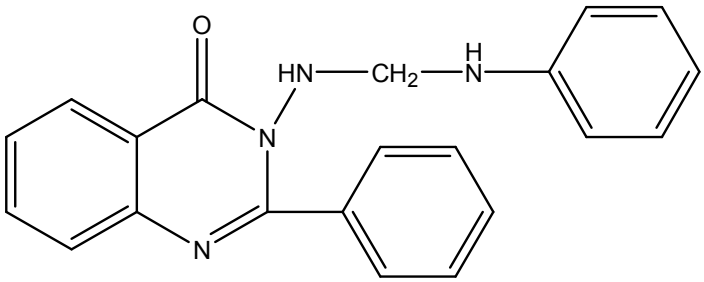
2-phenyl-3{[(piperazinyl) N-methyl] amino}- quinazolin-4(3H)-one

S4



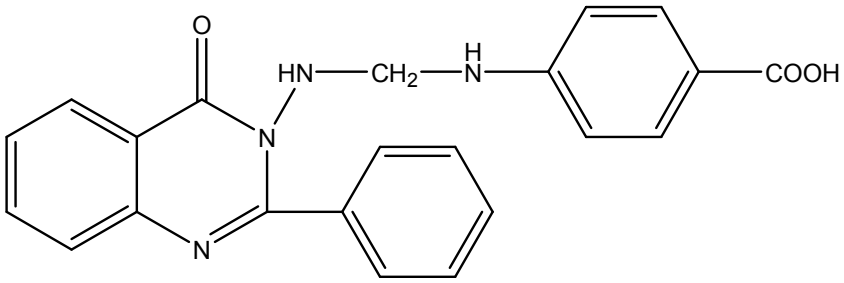
3{[(4-hydroxy phenyl) amino] methyl}amino -2-phenyl quinazolin-4(3H)-one

S5



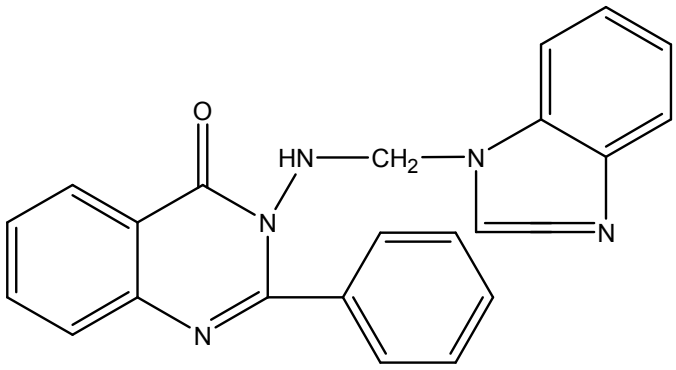
2-phenyl-3{[(phenyl amino) methyl]amino}-quinazolin-4(3H)-one

S6



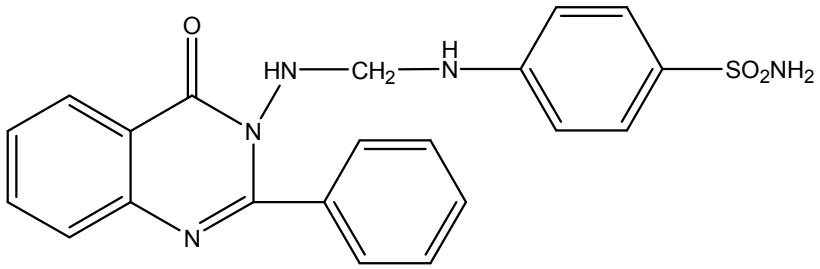
4-{[(2-phenyl 4-oxoquinazolin-3(4)-4-yl) amino] methyl}amino benzoic acid

S7



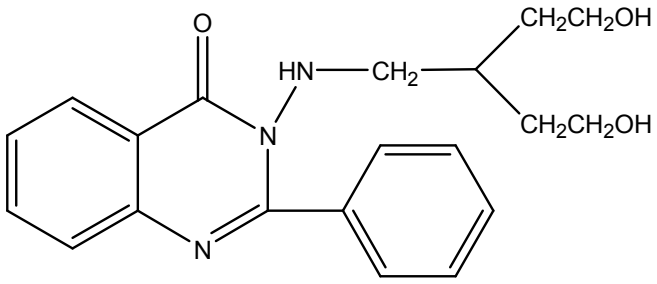
3-{[(1H-benzimidazol -1-yl-methyl) amino] 2-phenyl}quinazolin-4(3H)-one

S8



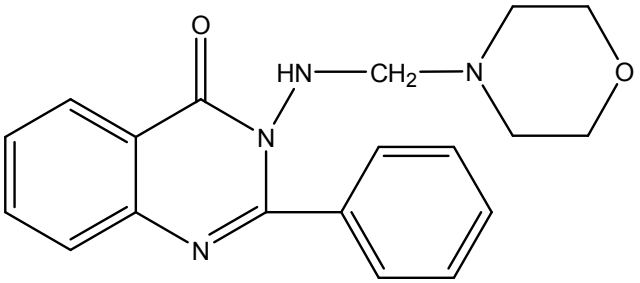
4-{[(2-pheyl-4-oxoquinazolin-3(4H)-yl) amino] methyl amino} benzene sulphonanmide

S9



2-phenyl -3-{[(diethanol amino) methyl] amino}quinazolin-4(3H)-one

S10



2-phenyl -3-{[(morpholinyl)N-methyl] amino}quinazolin-4(3H)-one

Chapter-III

Chapter III

Experimental Work

Synthesis

Step: I

Synthesis of 2-phenyl-3H-quinazolin-4-one

Chemicals required:

Anthranilic acid

Benzoyl chloride

Pyridine

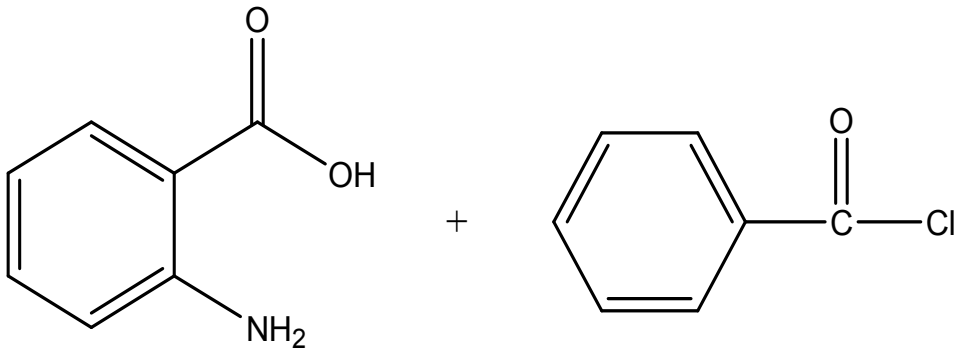
Ethanol

Procedure:

Anthranilic acid (1 mol) was treated with Benzoyl chloride (1 mol) in presence of pyridine and stirred for 3 hours and the resulting mixture was treated with 5% $NaHCO_3$ solution to get 2-phenyl benzoxazine 4-one and the precipitate was filtered and recrystallized from ethanol.

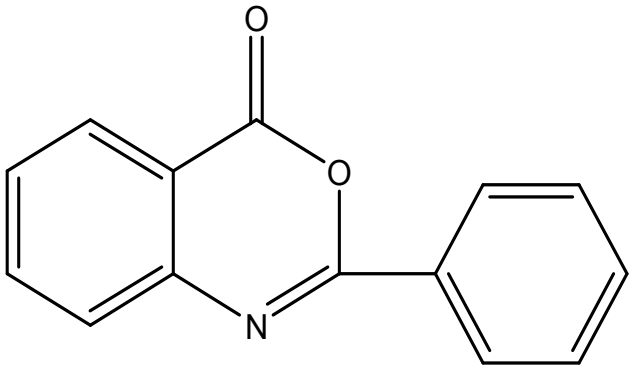
Synthesis of 2-phenyl-3H-quinazolin-4-one

Step: I



Anthranilic acid **Benzoyl Chloride**

Reflux for 3 hours | Pyridine



2-Phenyl-3,4-dihydro benzoxazin-4-one

Synthesis:

Step II:

Synthesis of 3-amino-2-phenyl-3H-quinazolin-4-one

Chemicals required:

Pyridine

Hydrazine hydrate

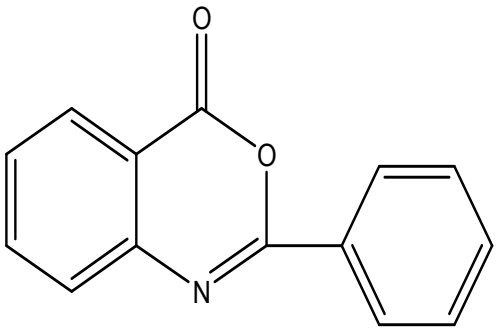
Ethanol

Procedure:

The solid obtained (1 mol) was treated with hydrazine hydrate (2 mol) in presence of ethanol and refluxed for 2-3 hours to form 2-phenyl 3-amino quinazolin-4-one. The content was cooled and the solid separated was filtered, washed well with water, dried and recrystallised from ethanol.

Synthesis of 3-amino-2-phenyl-3H-quinazolin-4-one

Step: II

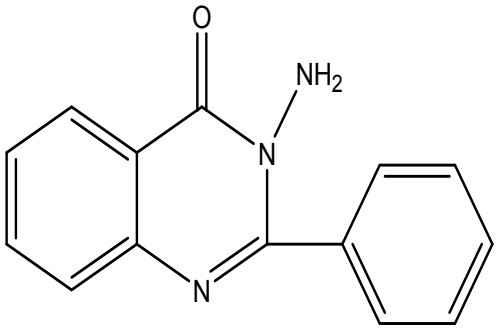


+ $NH_2 - NH_2 . H_2O$

Hydrazine hydrate

2-phenyl-3H-quinazolin-4one

Pyridine



3-amino-2-phenyl-3H-quinazolin-4-one

Synthesis

Step III

Synthesis of 3-substituted-2-phenyl-3H-quinazolin-4-one by Mannich reactions

Chemicals required:

3-amino-2-phenyl-3H-quinazolin-4-one - 0.01 mol

Formaldehyde - 0.15 mol

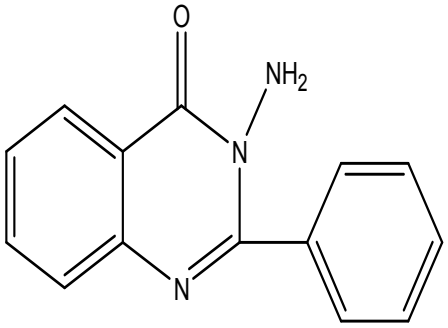
Amines - 0.01 mol

Ethanol - 20 ml

Procedure:

A mixture of 3-amino 2-phenyl-quinazolin-4(3H)-one (0.01 mol), amines (like Dicyclo hexylamine, Indole, piperazine, 4-aminophenol, aniline, PABA, Benzimidazole, Sulphanilamide, diethanolamine, morpholine (0.01 mol) and formaldehyde (0.15 mol) was taken in 20 ml of ethanol and heated under reflux for 4-5 hours. After coming to room temperature, the mixture was poured into crushed ice. Mannich base thus separated was filtered and recrystallized from ethanol. The purity of the compound was further confirmed on TLC using methanol : Chloroform : water (9:1:1) Iodine vapour was used for the detection of spots.

Reactions:

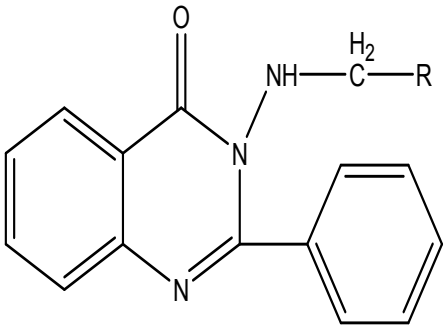


\+ HCHO + R' H

Formaldehyde **Amine containing compunds**

3-amino-2-phenyl-3H-quinazolin-4-one

Ethanol / reflux for 4 hours



2-phenyl-3{[(amino substituted) methyl] amino}-quinazolin -4(3H)-one

I Synthesis of 2-phenyl-3{[(dicyclohexylamino) methyl] amino}-quinazolin -4(3H)-one.

Chemicals required:

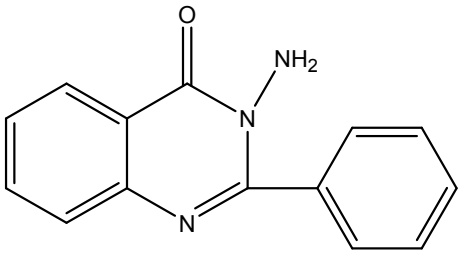
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Dicyclohexyl amine - 0.01 mol

Ethanol - 20 ml

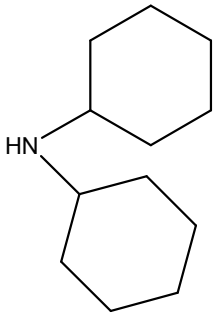
Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one

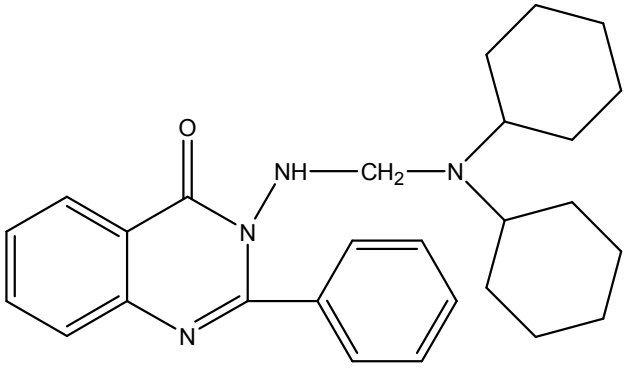
\+ HCHO +

Formaldehyde



Dicyclohenyl amine

Ethanol / reflux for 4 hours



2-phenyl-3{[(dicyclohenyl amino) methyl] amino}-quinazolin -4(3H)-one

II Synthesis of 2-phenyl-3{[(1H-indol-1-yl methyl) amino]}-quinazolin -4(3H)-one.

Chemicals required:

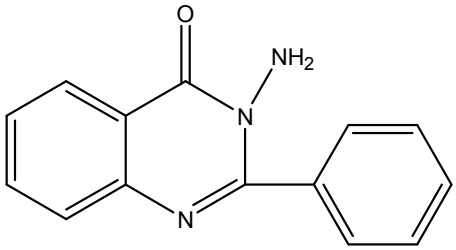
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Indole - 0.01 mol

Ethanol - 20 ml

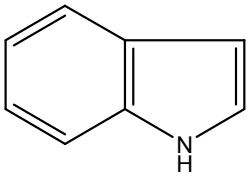
Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one

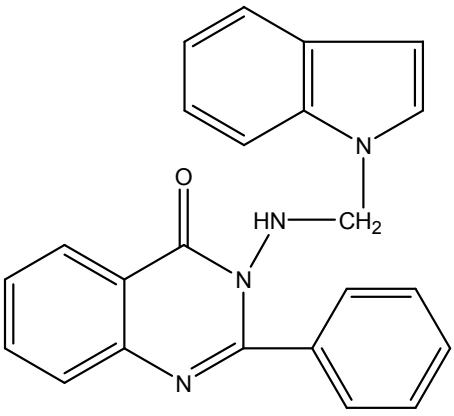
\+ HCHO +

Formaldehyde



Indole

Ethanol / reflux for 4 hours



2-phenyl-3{[(1H-indol-1-yl methyl) amino]}- quinazolin-4(3H)-one

III Synthesis of 2-phenyl-3{[(Piperazinyl) N-methyl] amino}-quinazolin -4(3H)-one.

Chemicals required:

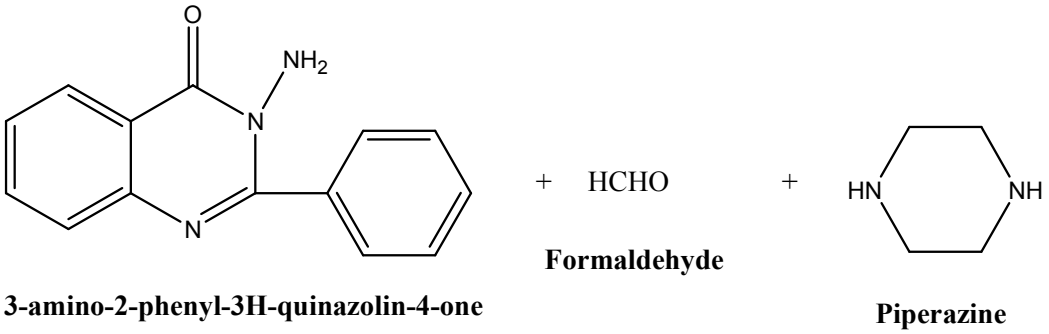
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Piperazine - 0.01 mol

Ethanol - 20 ml

Reactions:

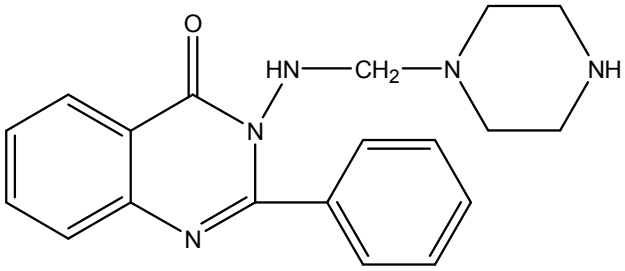


3-amino-2-phenyl-3H-quinazolin-4-one

Formaldehyde

Piperazine

Ethanol / reflux for 4 hours



2-phenyl-3{[(piperazinyl) N-methyl] amino}- quinazolin-4(3H)-one

IV Synthesis of 3-{[(4-hydroxy phenyl) amino] methyl} amino-2-phemyl quinazolin-4(3H)-one.

Chemicals required:

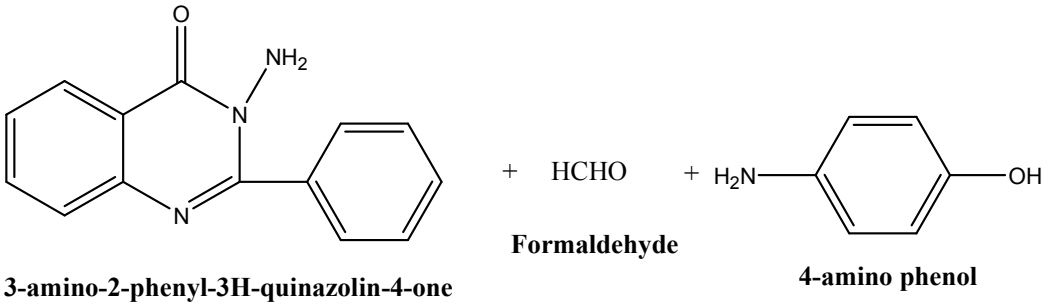
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

4-aminophenol - 0.01 mol

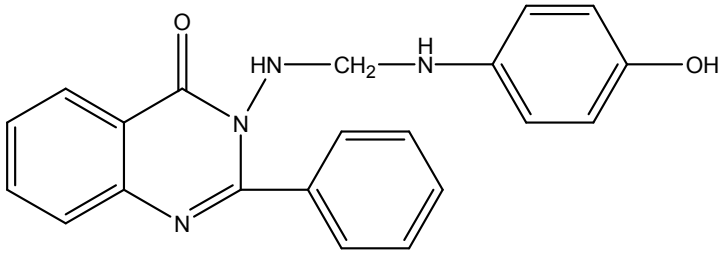
Ethanol - 20 ml

Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one **Formaldehyde** **4-amino phenol**

Ethanol / reflux for 4 hours



3{[(4-hydroxy phenyl) amino] methyl}amino -2-phenyl quinazolin-4(3H)-one

V Synthesis of 2-phenyl-3{[(phenyl amino) methyl] amino}-quinazolin -4(3H)-one.

Chemicals required:

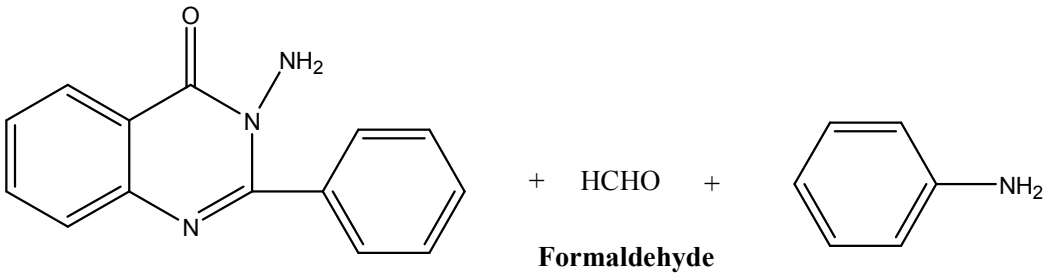
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Aniline - 0.01 mol

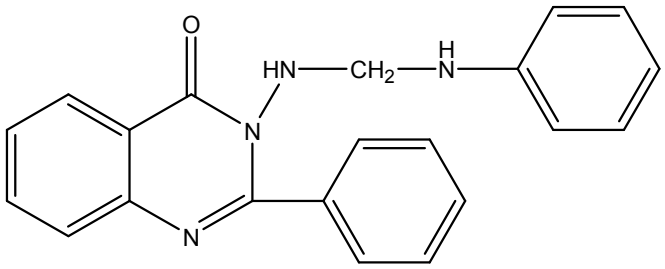
Ethanol - 20 ml

Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one **Formaldehyde** **Aniline**

Ethanol / reflux for 4 hours



2-phenyl-3{[(phenyl amino) methyl]amino}-quinazolin-4(3H)-one

VI Synthesis of 4-{[(2-phenyl-4-oxoquinazolin-3(4H)-yl) amino] methyl} amino benzoic acid.

Chemicals required:

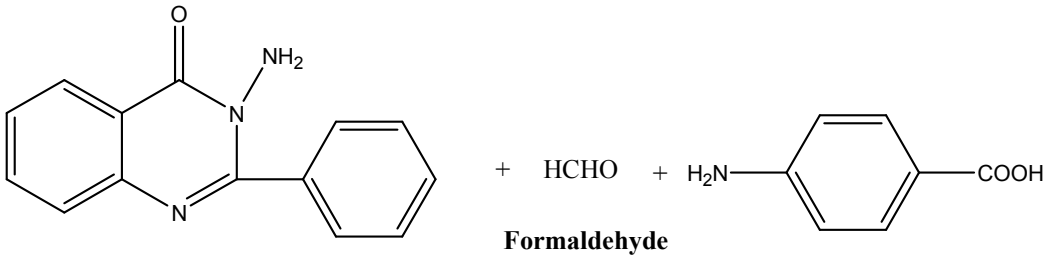
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

P-amino benzoic acid - 0.01 mol

Ethanol - 20 ml

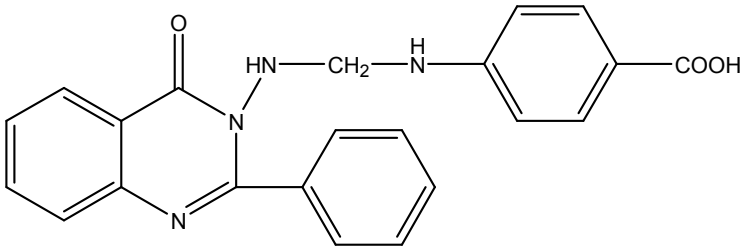
Reactions:



Formaldehyde

3-amino-2-phenyl-3H-quinazolin-4-one **P-amino benzoic acid**

Ethanol / reflux for 4 hours



4-{[(2-phenyl 4-oxoquinazolin-3(4)-4-yl) amino] methyl}amino benzoic acid

VII Synthesis of 3-{[(1H-benzimidazol-1-yl methyl) amino]}-2-phenyl-quinazolin -4(3H)-one.

Chemicals required:

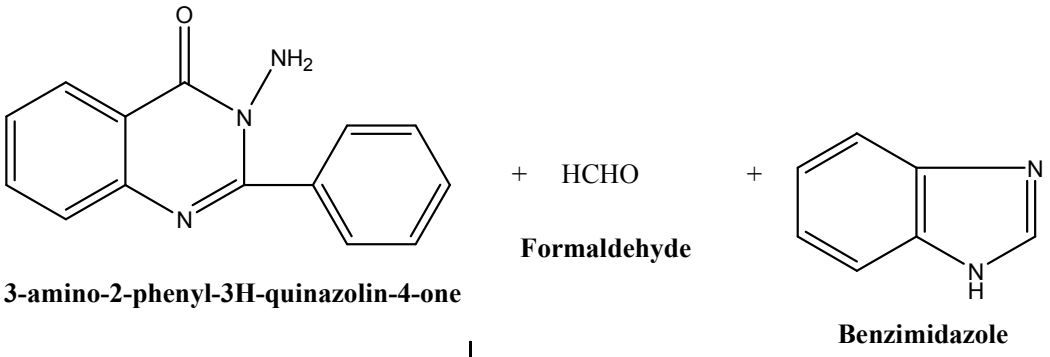
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Benzimidazole - 0.01 mol

Ethanol - 20 ml

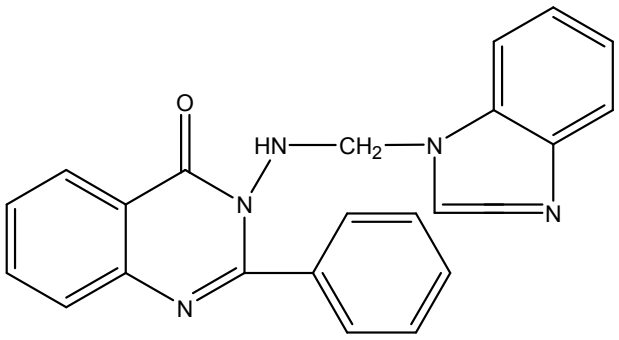
Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one

Formaldehyde

Benzimidazole



3-{[(1H-benzimidazol -1-yl-methyl) amino] 2-phenyl}quinazolin-4(3H)-one

VIII Synthesis of 4-{[(2-phenyl-4-oxoquinazolin-3(4H)-yl) amino] methyl} amino benzene sulphonamide.

Chemicals required:

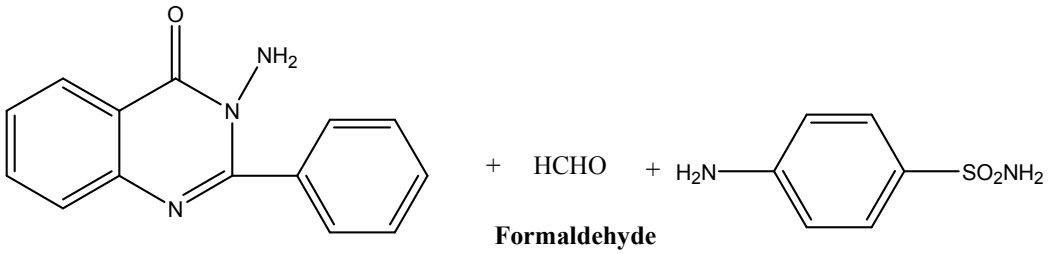
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Sulphanilamide - 0.01 mol

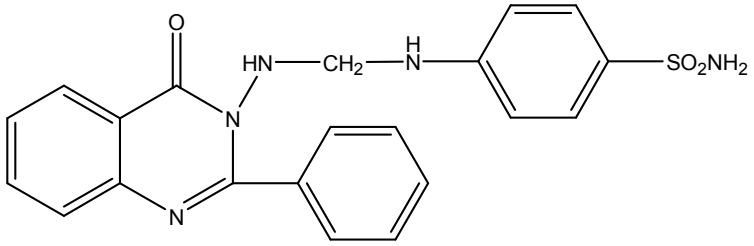
Ethanol - 20 ml

Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one **Formaldehyde** **Sulphanilamide**

Ethanol / reflux for 4 hours



4-{[(2-pheyl-4-oxoquinazolin-3(4H)-yl) amino] methyl amino} benzene sulphonanmide

IX Synthesis of 2-phenyl-3{[(diethanol amino) methyl] amino}-quinazolin -4(3H)-one.

Chemicals required:

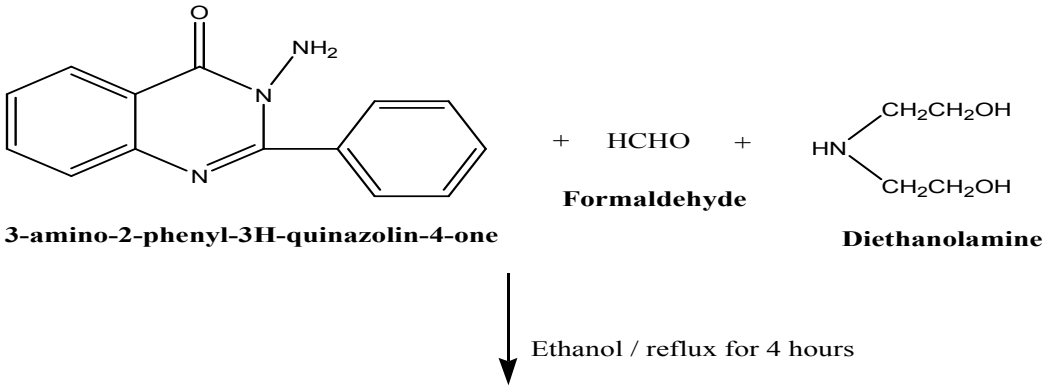
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

Diethanolamine - 0.01 mol

Ethanol - 20 ml

Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one **Diethanolamine**

2-phenyl -3-{[(diethanol amino) methyl] amino}quinazolin-4(3H)-one

X Synthesis of 2-phenyl-3{[(Morpholinyl) N-methyl] amino]}-quinazolin -4(3H)-one.

Chemicals required:

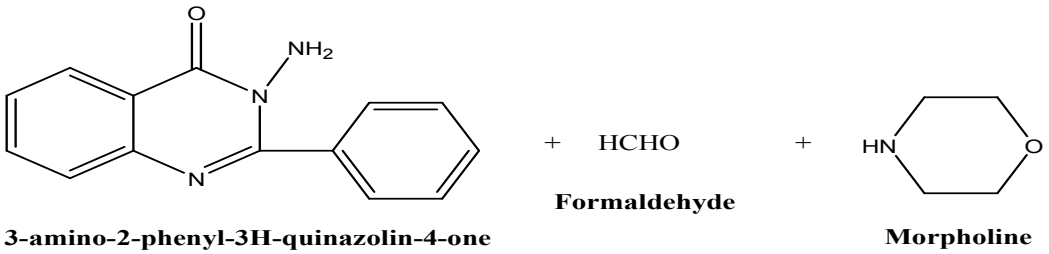
3-amino-2-phenyl quinazolin-4(3H)-one - 0.01 mol

Formaldehyde - 0.15 mol

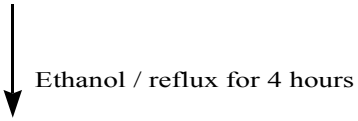
Morpholine - 0.01 mol

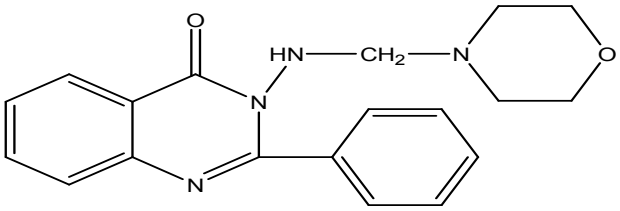
Ethanol - 20 ml

Reactions:



3-amino-2-phenyl-3H-quinazolin-4-one **Morpholine**





2-phenyl -3-{[(morpholinyl)N-methyl] amino}quinazolin-4(3H)-one

Chapter-IV

Chapter IV

Table No. 1-Physical data of titled compounds

Code	Chemical name	M.F	M.Wt.	yield	Appearance
S1	2-phenyl-3{[(dicyclohexylamino) methyl] amino}-quinazolin -4(3H)-one	$C_{27}H_{34}N_4O$	430	91.55%	Black gummy solid
S2	2-phenyl-3{[(1H-indol-1-yl methyl) amino]}-quinazolin -4(3H)-one.	$C_{23}H_{18}N_4O$	366	78.37%	Black coloured Solid
S3	2-phenyl-3{[(Piperazinyl) N-methyl] amino}-quinazolin -4(3H)-one	$C_{19}H_{21}N_2O$	335	89.33%	Pale yellow coloured Solid
S4	3-{[(4-hydroxy phenyl) amino] methyl} amino-2-phemyl quinazolin-4(3H)-one	$C_{21}H_{18}N_4O_2$	358	81.22%	Brown coloured crystals
S5	2-phenyl-3{[(phenyl amino) methyl] amino}-quinazolin -4(3H)-one	$C_{21}H_{18}N_4O$	342	75.65%	Yellowish crystals
S6	4-{[(2-phenyl-4-oxoquinazolin-3(4H)-yl) amino] methyl} amino benzoic acid	$C_{22}H_{18}N_4O_3$	386	83.37%	Pale brown coloured Solid
S7	3-{[(1H-benzimidazol-1-yl methyl) amino]}-2-phenyl-quinazolin -4(3H)-one	$C_{22}H_{17}N_5O$	367	90.05%	Black coloured Solid
S8	4-{[(2-phenyl-4-	$C_{21}H_{19}N_5O_3S$	421	78.84%	Pale yellow

	oxoquinazolin-3(4H)-yl) amino] methyl} amino benzene sulphonamide				coloured Solid
S9	2-phenyl-3{[(diethanol amino) methyl] amino}-quinazolin -4(3H)-one	$C_{19}H_{22}N_4O_3$	354	85.39%	Black coloured crystals
S10	2-phenyl-3{[(Morpholinyl) N-methyl] amino]}-quinazolin -4(3H)-one.	$C_{19}H_{20}N_4O_2$	336	91.03%	Black coloured Solid

Melting point analysis:

Melting point was found in an open end capillary tube method by electrically heating melting point apparatus.

The melting point synthesized compounds.

Table No.2

S. No.	Compound Code	Melting point (°C)
1	S1	156
2	S2	137
3	S3	168
4	S4	159
5	S5	148
6	S6	125
7	S7	124
8	S8	149
9	S9	135
10	S10	141

Table: No .3

Elemental Composition Analysis (%)					
Code	**C**	**H**	**N**	**O**	**S**
S1	75.31	7.96	13.01	3.72	-
S2	75.39	4.95	15.29	4.37	-
S3	68.04	6.31	20.88	4.77	-
S4	70.38	5.06	15.63	8.93	-
S5	73.67	5.3	16.36	4.67	-
S6	68.38	4.7	14.5	12.42	
S7	71.92	4.66	19.06	4.35	-
S8	59.84	4.54	16.62	11.39	7.61
S9	64.39	6.26	15.81	13.54	-
S10	67.84	5.99	16.66	9.51	-

Thin Layer Chromatography Analysis

Thin layer chromatography analysis was carried out by using silica gel G (0.5 mm thickness) coated over glass plate (12 x 20 cm) as stationary phase, Methanol : Chloroform : Water (9:1:1) as mobile phase, the spots were visualized by iodine vapours.

R_f value of the synthesized compounds

Table No.4

S. No.	Compound Code	R_f Value
1	S1	0.9305
2	S2	0.8667
3	S3	0.9054
4	S4	0.8714
5	S5	0.9577
6	S6	0.8933
7	S7	0.8472
8	S8	0.9459
9	S9	0.8875
10	S10	0.9206

Chapter-V

Chapter V

Infrared Spectral Analysis

The structure of the synthesized compounds was elucidated by PERKIN-ELMER FT-IR spectrophotometry using Potassium bromide disc. The Infrared values were measured as wave number in cm^{-1} and the results are shown below.

Compound S1

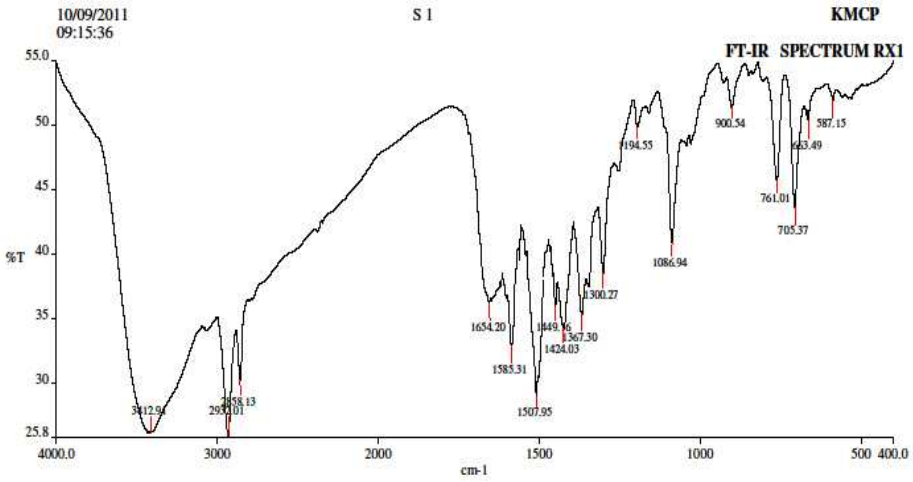
IR Values

3412 (NH, 2^0 amine)

2932 (CH str. aromatic)

2858 (CH str. alkyl)

1654 (C=O str. of quinazoline)



Spectrum Pathname: C:\PEL_DATA\SPECTRA\s1r.002

s1r.pk

s1r.002 3601 4000.00 400.00 25.94 55.03 4.00 %T 5 1.00

REF 4000 54.81 2000 47.76 600
3412.91 26.13 2932.01 25.94 2858.13 30.27 1654.20 36.11 1585.31 32.83
1507.95 28.93 1449.16 35.93 1424.03 34.19 1367.30 35.32 1300.27 38.55
1194.55 49.85 1086.94 40.92 900.54 51.33 761.01 45.72 705.37 43.59
663.49 50.41 587.15 51.98

Compound S2

IR Values

3238 (NH, 2^0 amine)

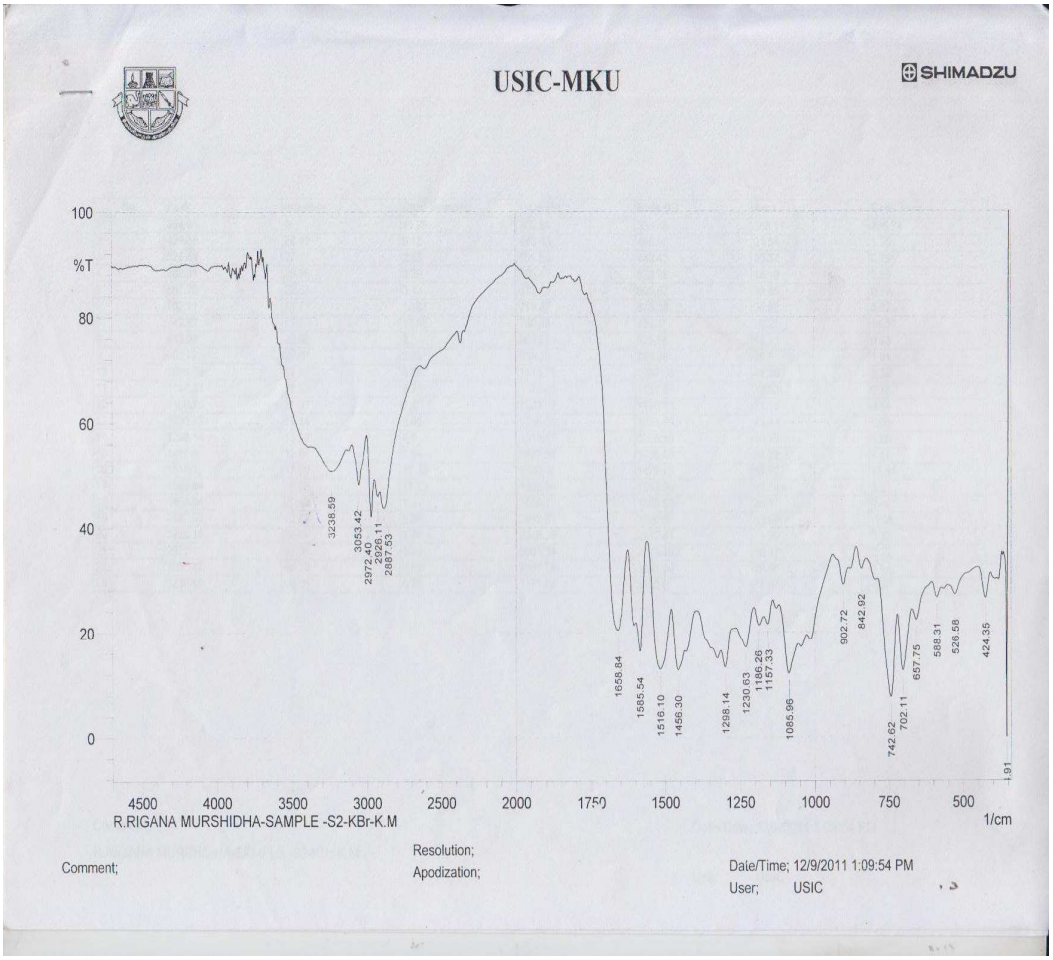
3053 (CH str. aromatic)

2972 (CH str. alkyl)

1658 (C=O str. of quinazoline)

1585, 1456 (C-N str.)

742 (CH bending, 4-substituted benzene)



Compound S3

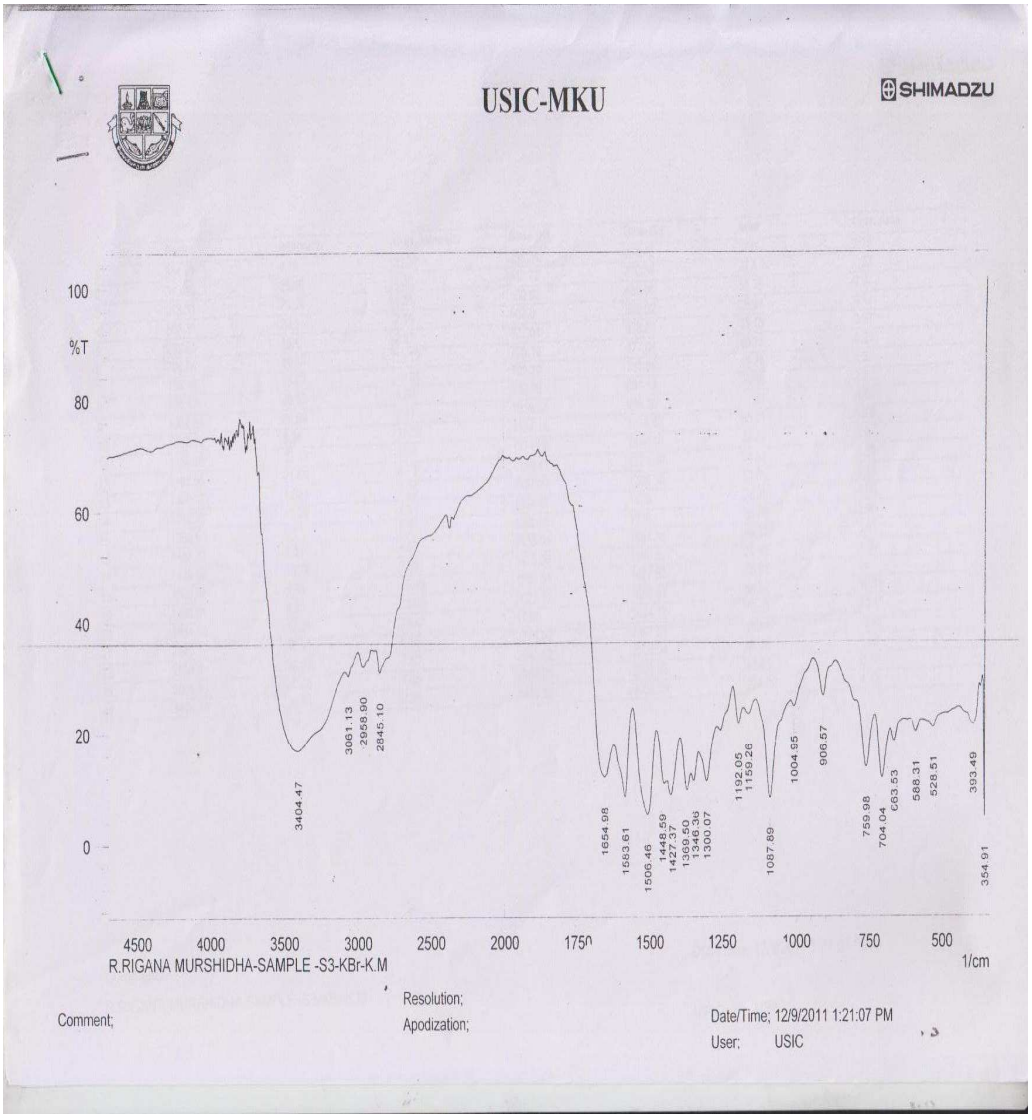
IR Values

3404 (NH, 2^0 amine)

3061 (CH str. aromatic)

1654 (C=O str. of quinazoline)

1583 (C-N str.)



Compound S4

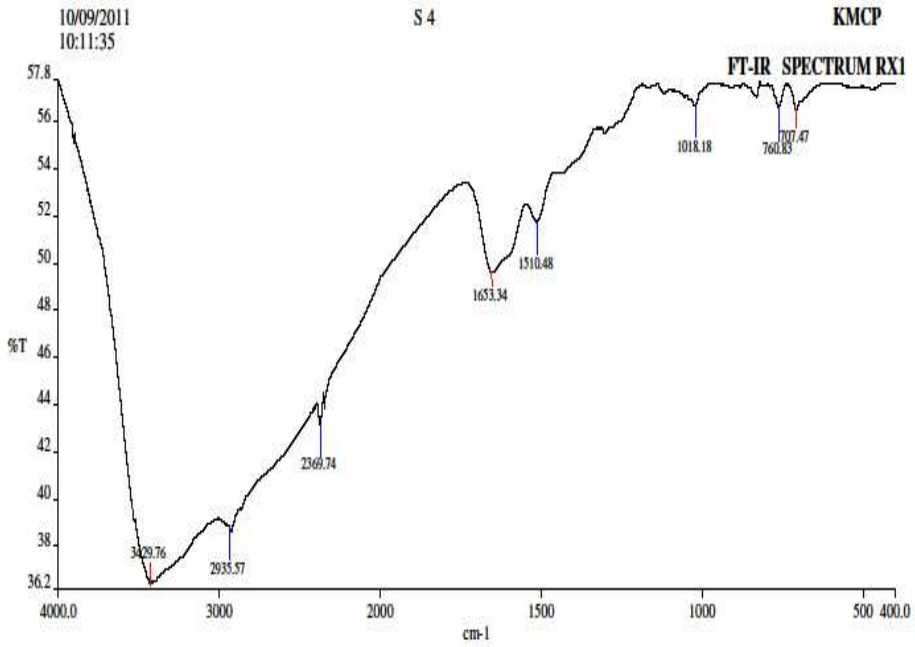
IR Values

3429 (OH str., phenolic)

2935 (CH str. aromatic)

1653 (C=O str. of quinazoline)

1510 (C-N str.)



Spectrum Pathname: C:\PEL_DATA\SPECTRA\s4r.002

s4r.pk

s4r.002 3601 4000.00 400.00 36.34 57.75 4.00 %T 5 1.00

REF 4000 57.75 2000 49.38 600
3429.76 36.34 1653.34 49.59 707.47 56.51

Compound S5

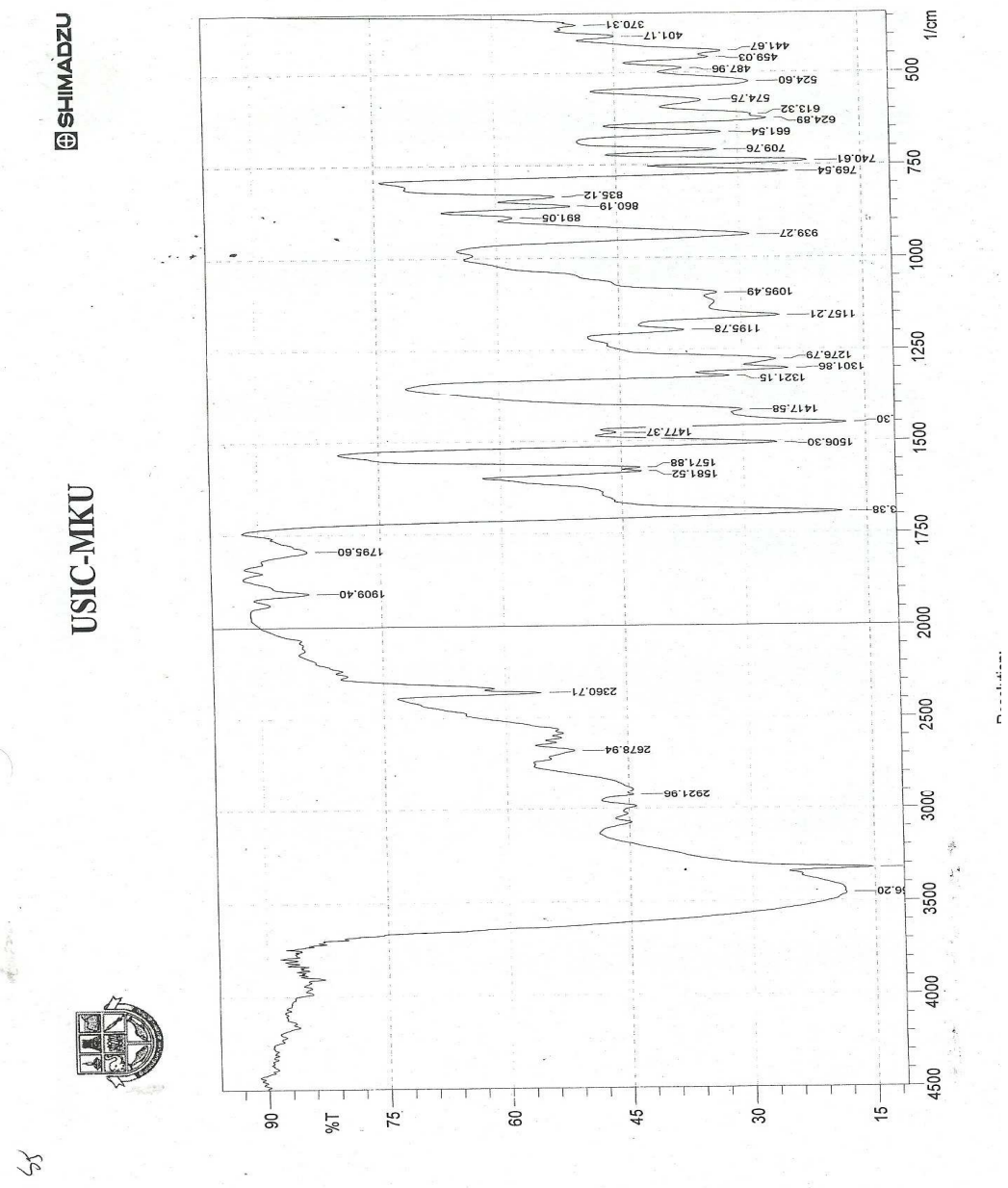
IR Values

3323 (NH, 2^0 amine)

2921 (CH str. aromatic)

1693 (C=O str. of quinazoline)

1571, 1477 (C-N str.)



Compound S6

IR Values

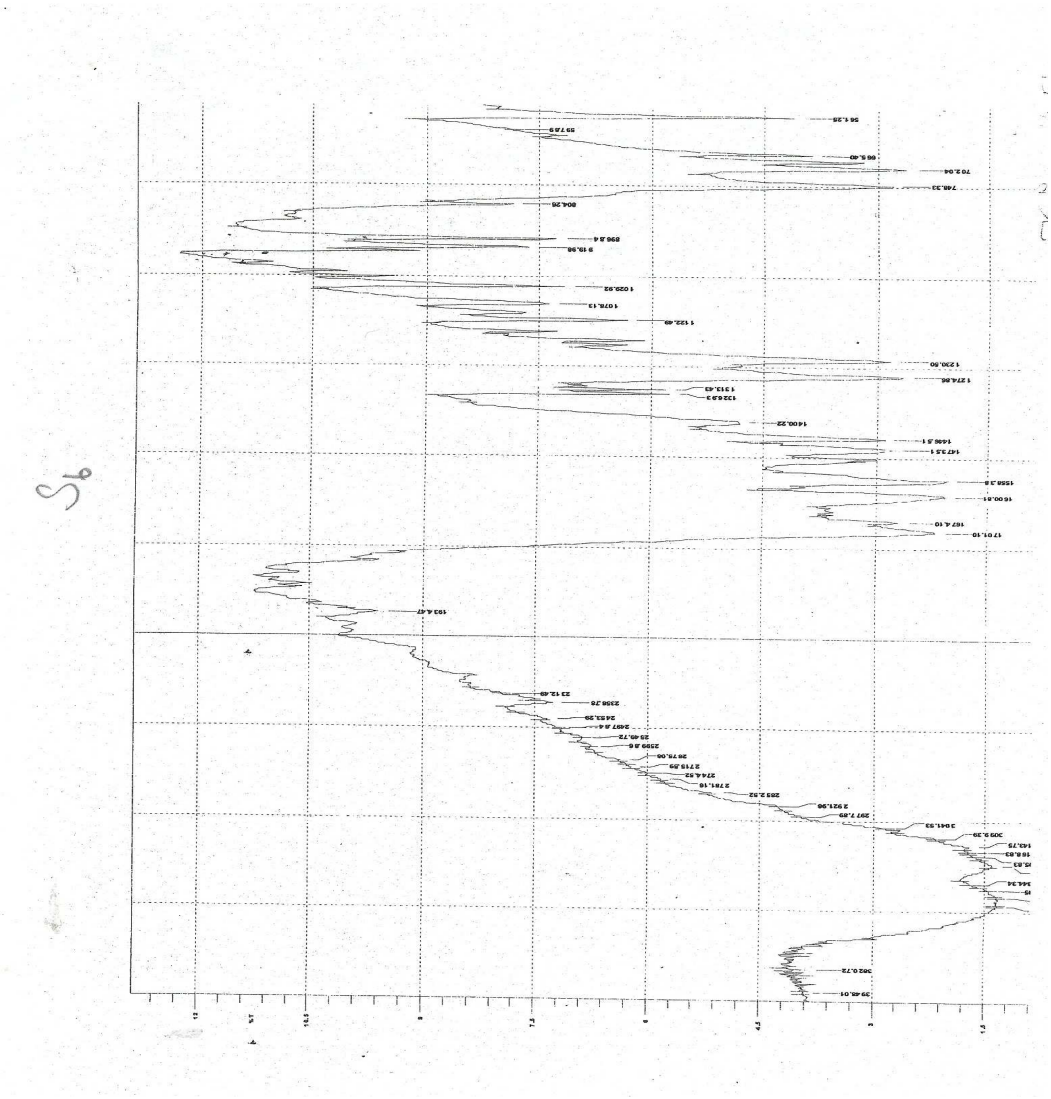
3344 (NH, 2^0 amine)

2977 (CH str. alkyl)

1701 (C=O str. carboxylic acid)

1674 (C=O str. of quinazoline)

1230 (C-O str.)



Compound S7

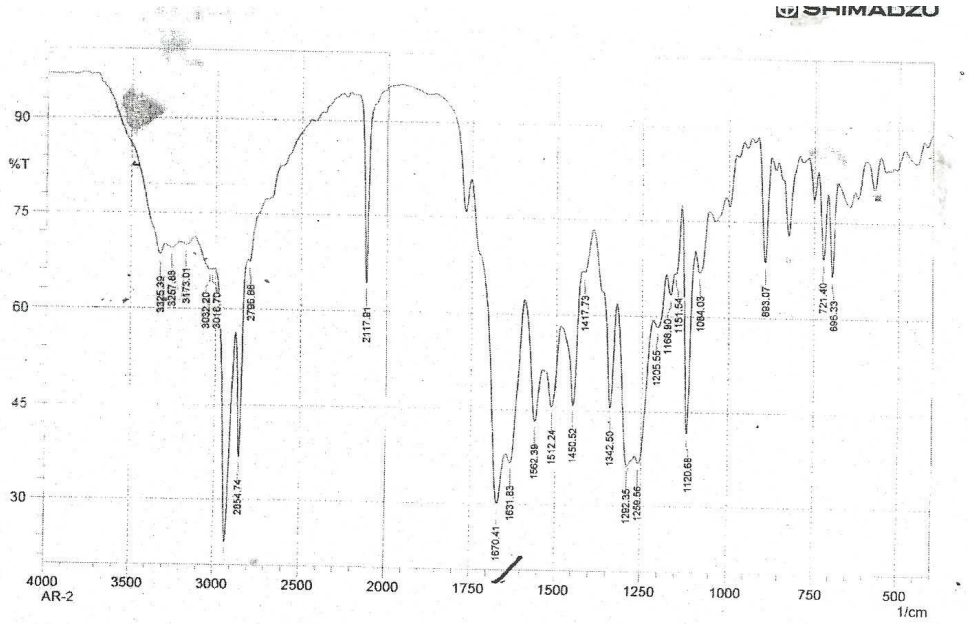
IR Values

3325 (NH, 2^0 amine)

3032 (CH str. aromatic)

1670 (C=O str. of quinazoline)

1562. 1450 (C-N str.)



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	696.33	66.551	13.748	707.9	682.82	3.375	1.005
2	721.4	69.305	11.783	736.83	709.83	3.401	0.958
3	893.07	68.603	18.24	914.29	875.71	4.069	1.718
4	1084.03	66.911	5.284	1095.6	1058.96	5.646	0.739
5	1120.68	41.488	33.105	1136.11	1097.53	9.164	4 03
6	1151.54	66.593	2.366	1155.4	1138.04	2.662	0.203
7	1168.9	63.297	3.224	1178.55	1157.33	3.999	0.246
8	1205.55	58.327	3.343	1217.12	1180.47	7.921	0.584
9	1259.56	36.839	5.26	1269.2	1219.05	17.28	1.395
10	1292.35	36.367	11.335	1323.21	1271.13	19.275	2.711
11	1342.5	45.455	19.372	1392.65	1325.14	15.055	3.406
12	1417.73	66.713	1.054	1421.58	1394.58	4.266	0.129
13	1450.52	45.845	17.423	1487.17	1423.51	16.059	3.118
14	1512.24	45.532	9.523	1539.25	1489.1	14.972	1.877
15	1562.39	43.164	12.632	1593.25	1541.18	15.379	2.409
16	1631.83	36.56	7.973	1645.33	1595.18	17.168	1.717
17	1670.41	30.102	17.504	1751.42	1647.26	30.398	4.442
18	2117.91	64.348	30.006	2171.92	2025.32	8.216	4.587
19	2796.88	67.382	1.202	2806.52	2702.36	13.98	0.107
20	2854.74	36.734	23.583	2879.82	2808.45	19.327	4.583
21	2929.97	23.288	36.909	3003.27	2881.75	42.886	16.782
22	3018.7	66.102	0.012	3020.63	3005.2	2.771	0.001
23	3032.2	66.085	0.434	3119	3024.48	15.723	0.233
24	3173.01	69.936	0.782	3209.66	3120.93	13.529	0.218
25	3257.88	69.436	0.44	3273.31	3211.59	9.635	0.097
26	3325.39	68.431	3.729	3666.8	3294.53	31.22	1.273

Compound S8

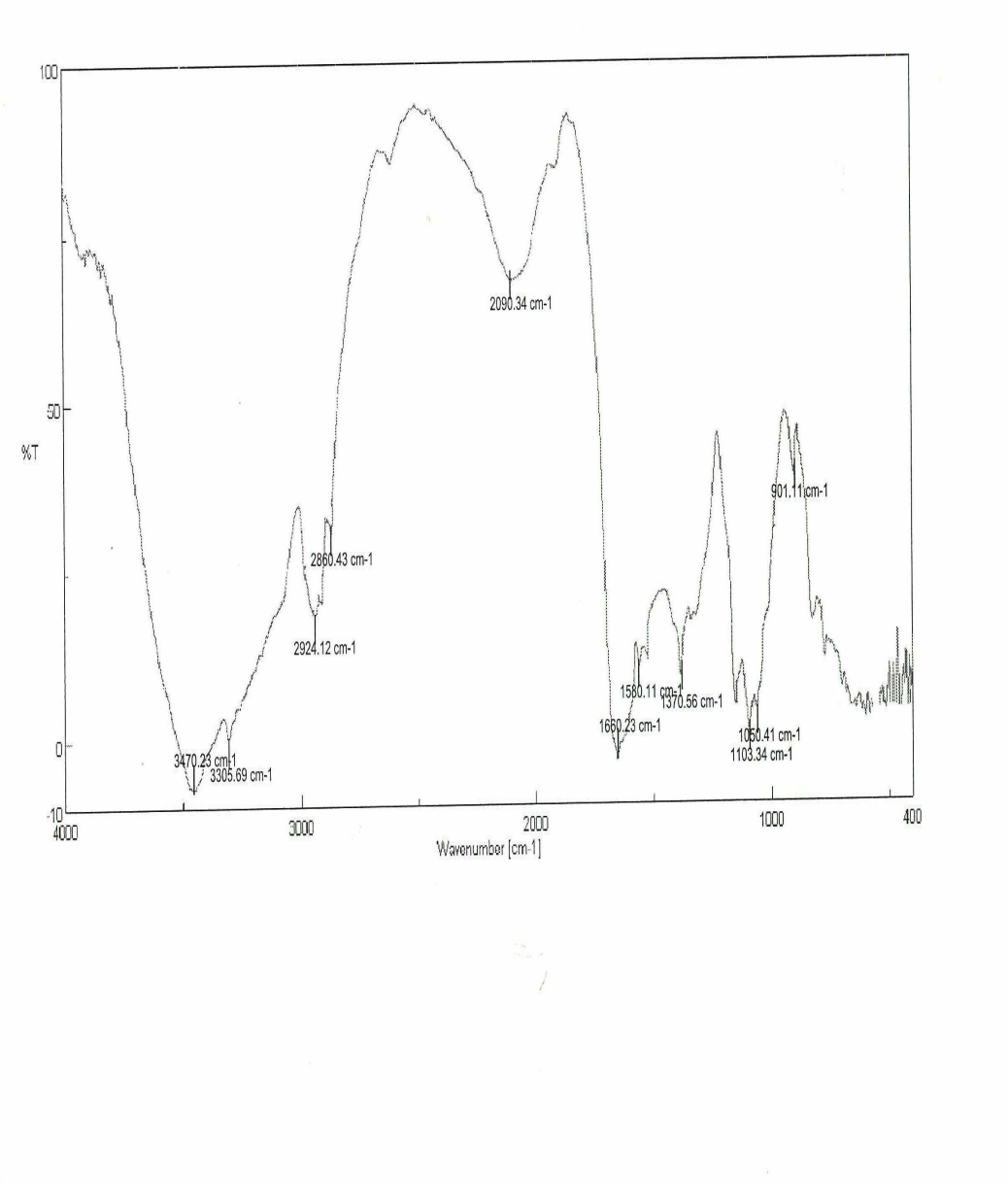
IR Values

3305.69 N-H stretch (in SO_2NH_2)

1660.23 C=O stretch

901.11 S-N stretch

1370.56 SO_2 stretch



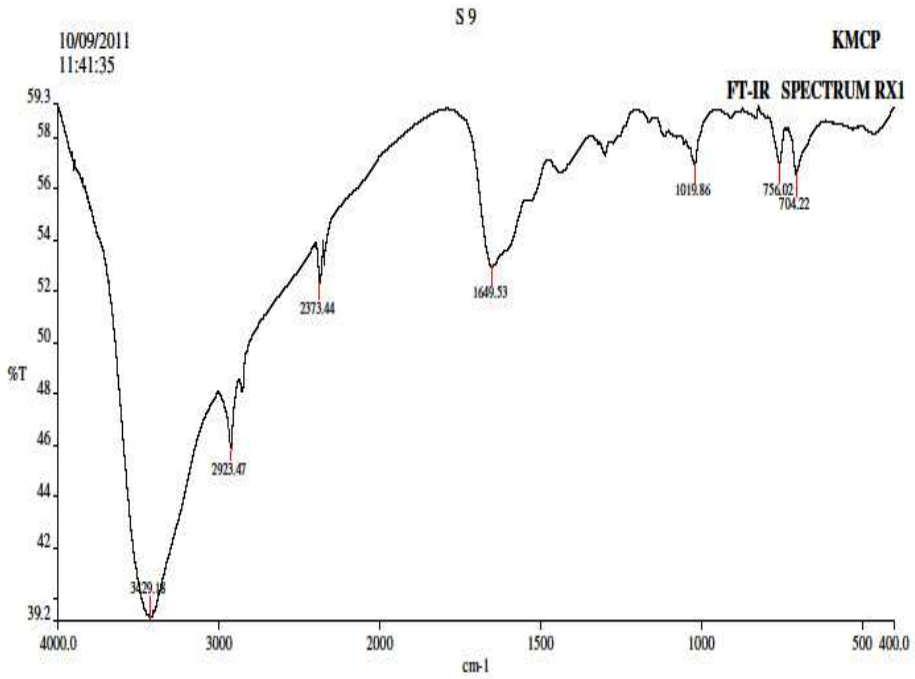
Compound S9

IR Values

2923 (CH str. aromatic)

1020 (C-O str.primary alcohol)

1649 (C=O str. of quinazoline)



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s9r.pk

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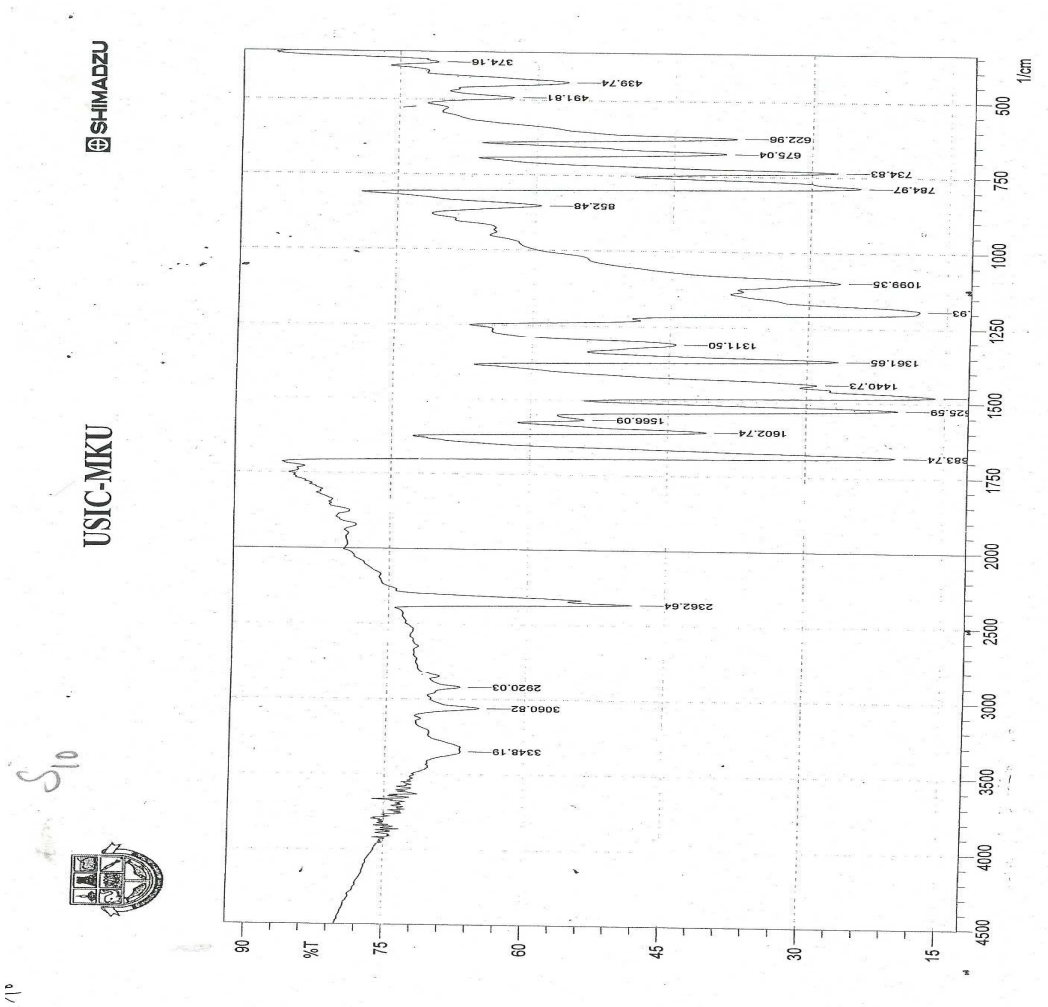
REF 4000 59.22 2000 57.29 600
3429.18 39.23 2923.47 45.88 2373.44 52.34 1649.53 52.99 1019.86 56.94
756.02 56.97 704.22 56.59

Compound S10

IR Values

3348	(NH, 2^0 amine)
3060	(CH str. aromatic)
2920	(CH str. alkyl)
1683	(C=O str. of quinazoline)
1566, 1440	(C-N str.)
1099	(C=O str)
1293	(C-O str.)



Nuclear Magnetic Resonance spectral analysis

The structure of the synthesized compounds were elucidated by BRUCKER 300 Mhz FT-NMR using TMS (Tetra Methyl Silane) as an internal standard. The proton magnetic resonance spectroscopic values are measured in δppm in $CDCl_3$.

Compound S1

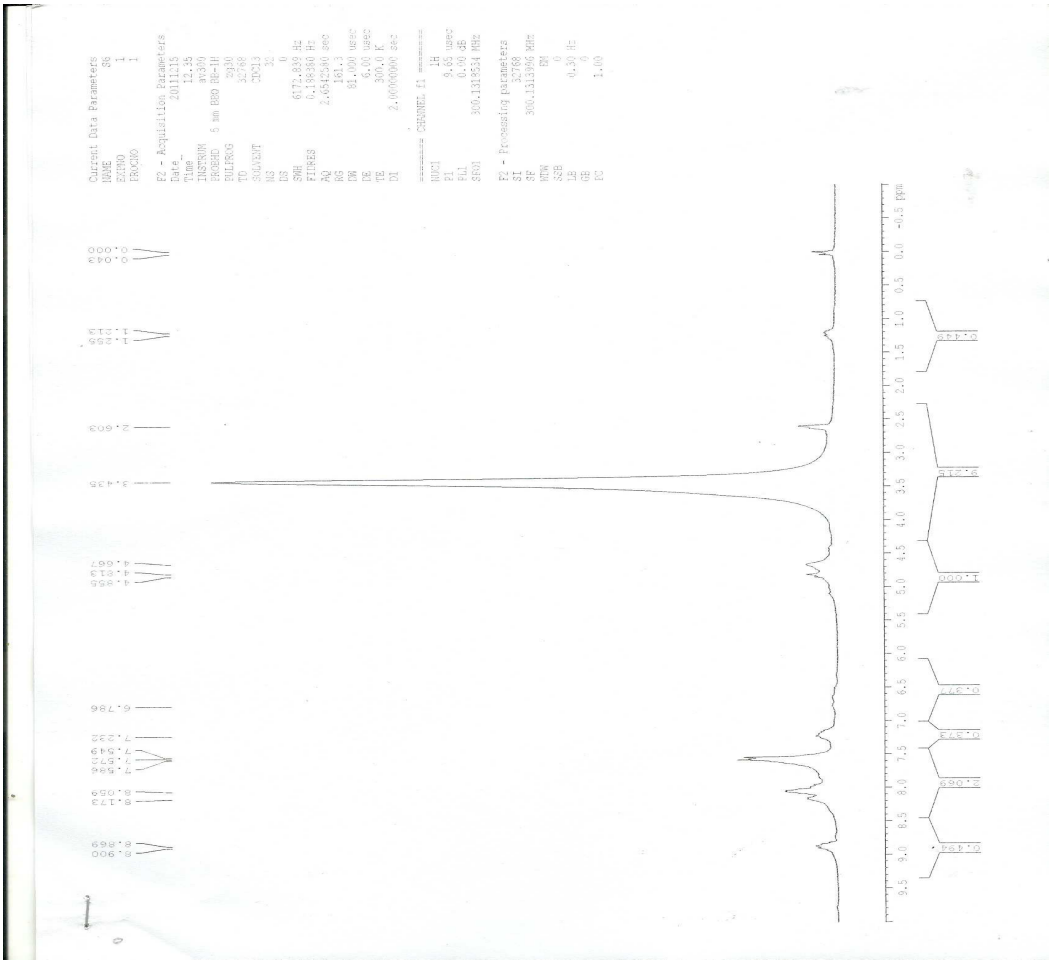
NMR Values

1.38-1.49 (m, 16H, CH_2 of cyclo hexane)

2.57 (s, 2H, -CH of cyclo hexane)

3.75 (s, 2H, -CH_2 of amino alkyl)

7.62-7.9 (m, 9H, ArH)



Compound S2

NMR Values

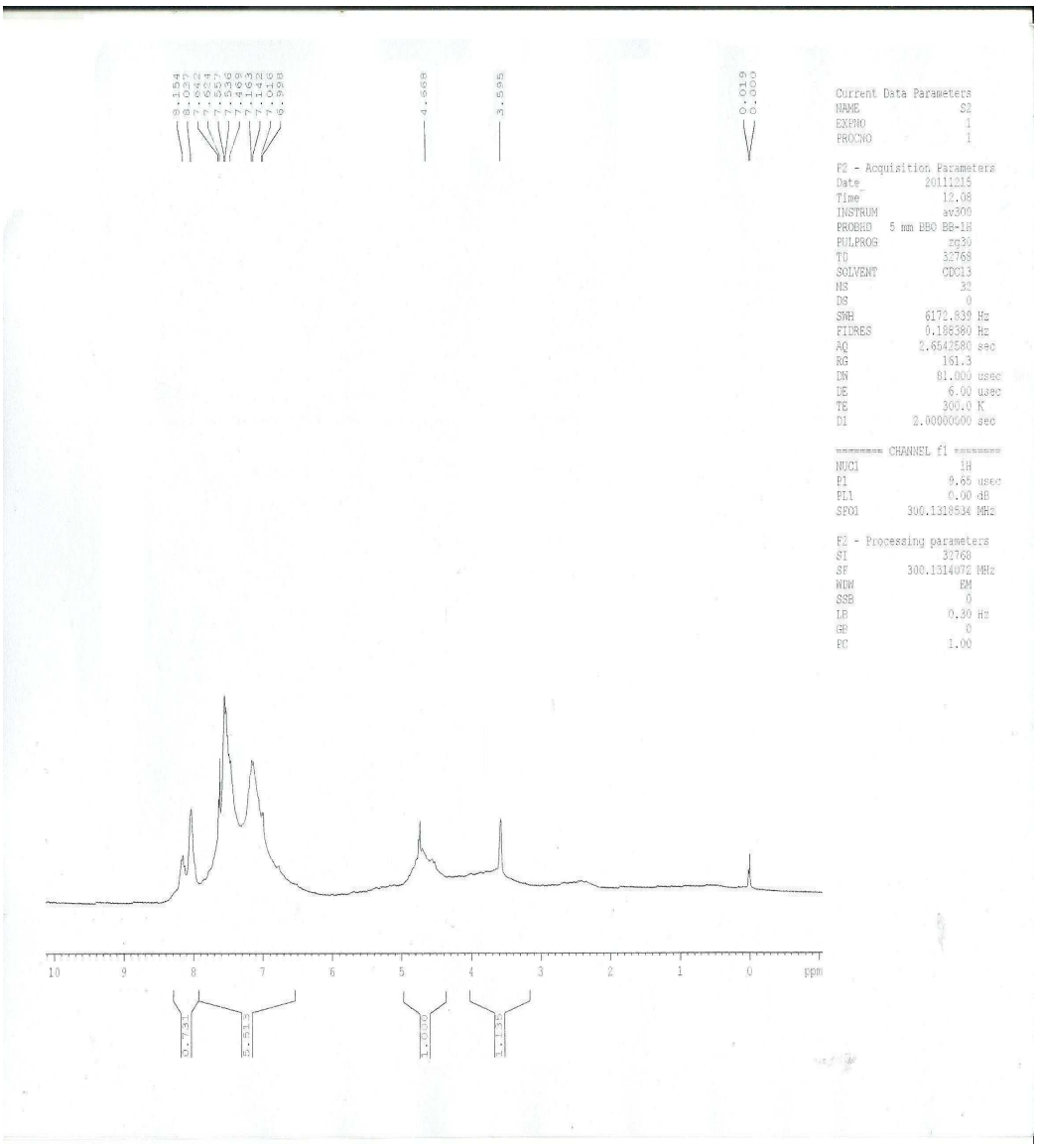
7.142-8.154 (m, 13H, ArH)

6.998 (s, 1H, CH_2 of (3) indole)

7.016 (s, 1H, CH_2 of (2) indole)

4.668 (s, 1H, -NH)

3.595 (s, 2H, CH_2)



Compound S3

NMR Values

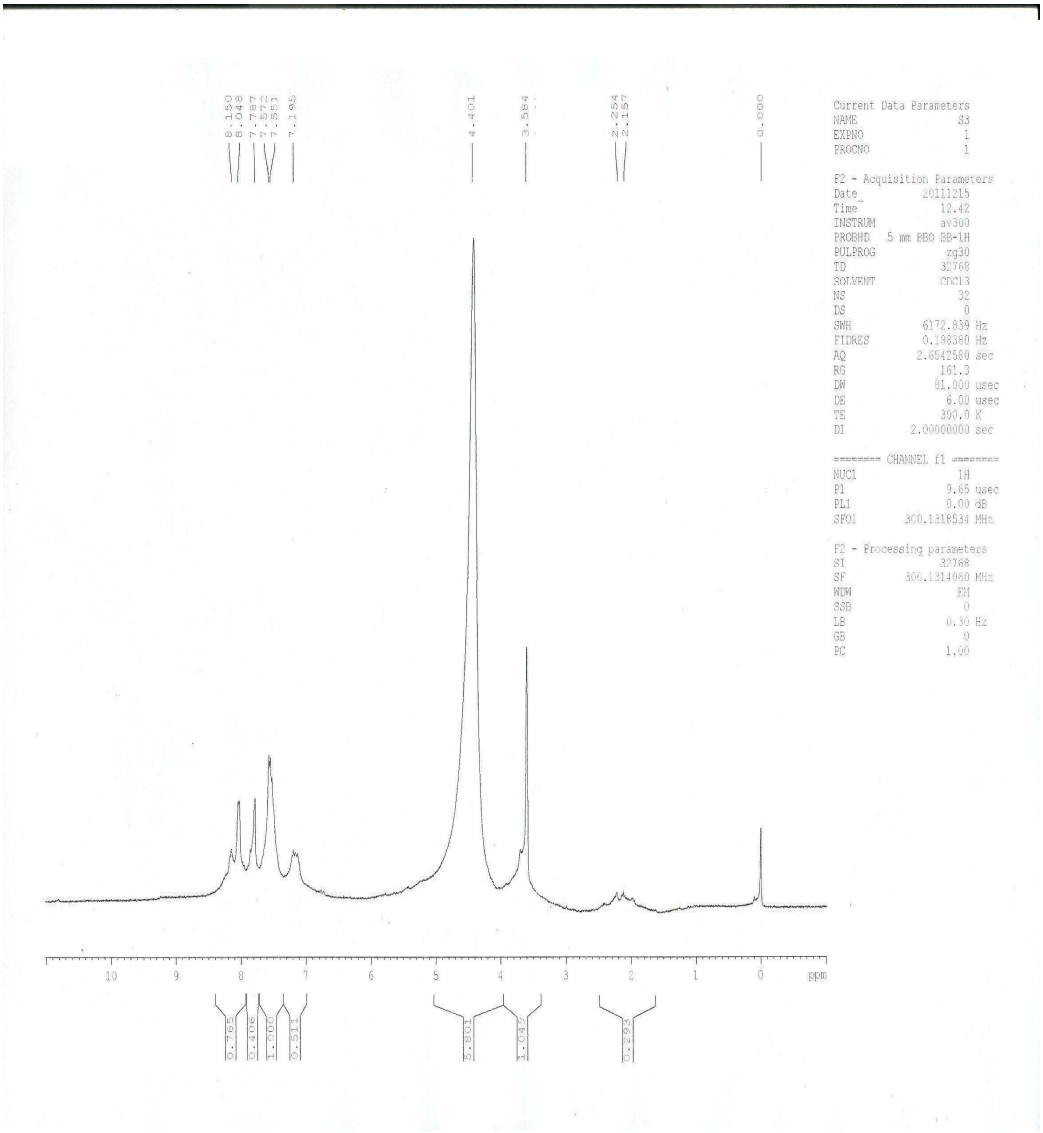
7.195-8.150 (m, 9H, ArH)

4.401 (s, 1H, -NH)

3.584 (s, 2H, $-CH_2$)

2.254 (s, 2H, CH_2 of (3) piperazine)

2.157 (s, 2H CH2 of (2) piperazine)



Compound S4

NMR Values

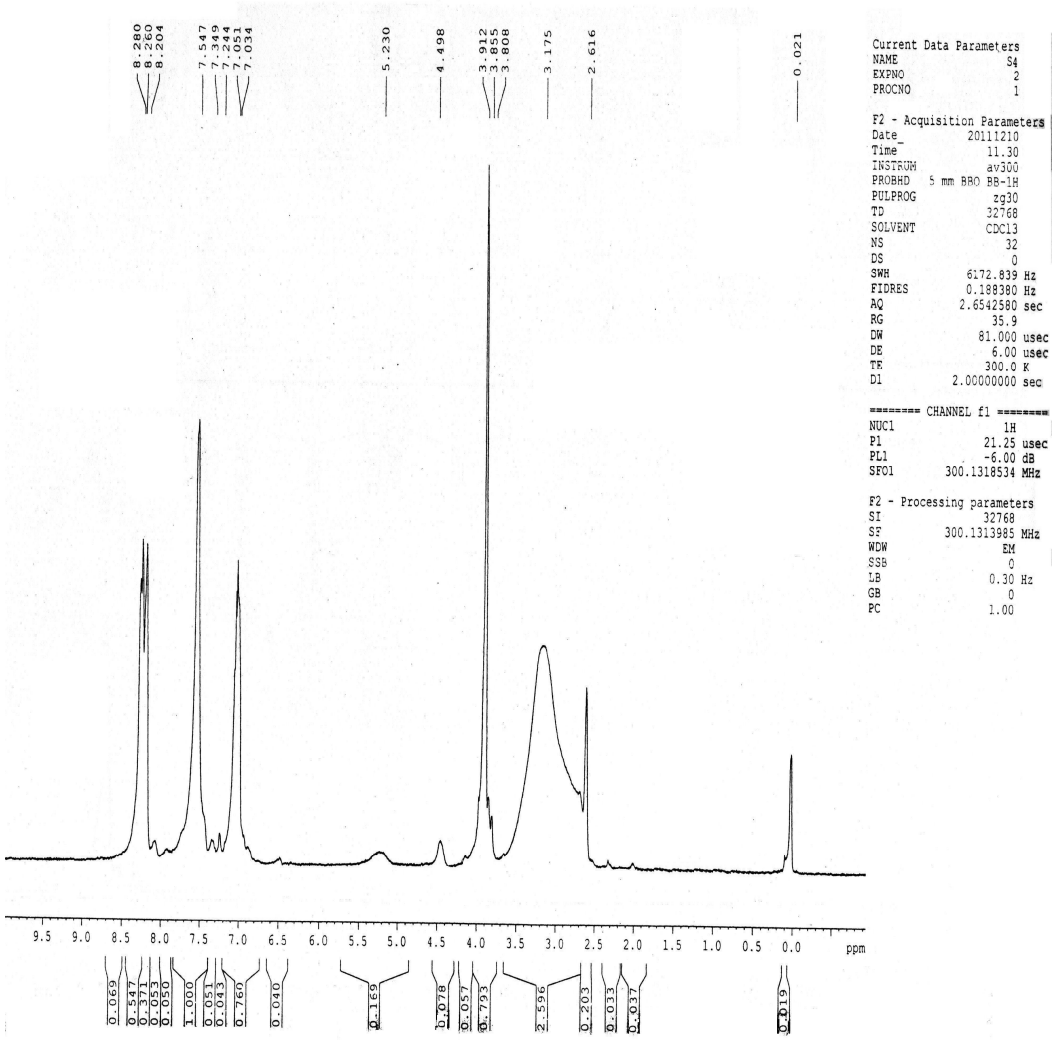
7.034- 8.280 (m, 14H, ArH)

5. 230 (s, 1H, -OH)

4.498 (s, 1H, -N-NH)

3.855 (s, 2H, -CH_2)

3.175 (s, 1H, -NH Ph)



Compound S5

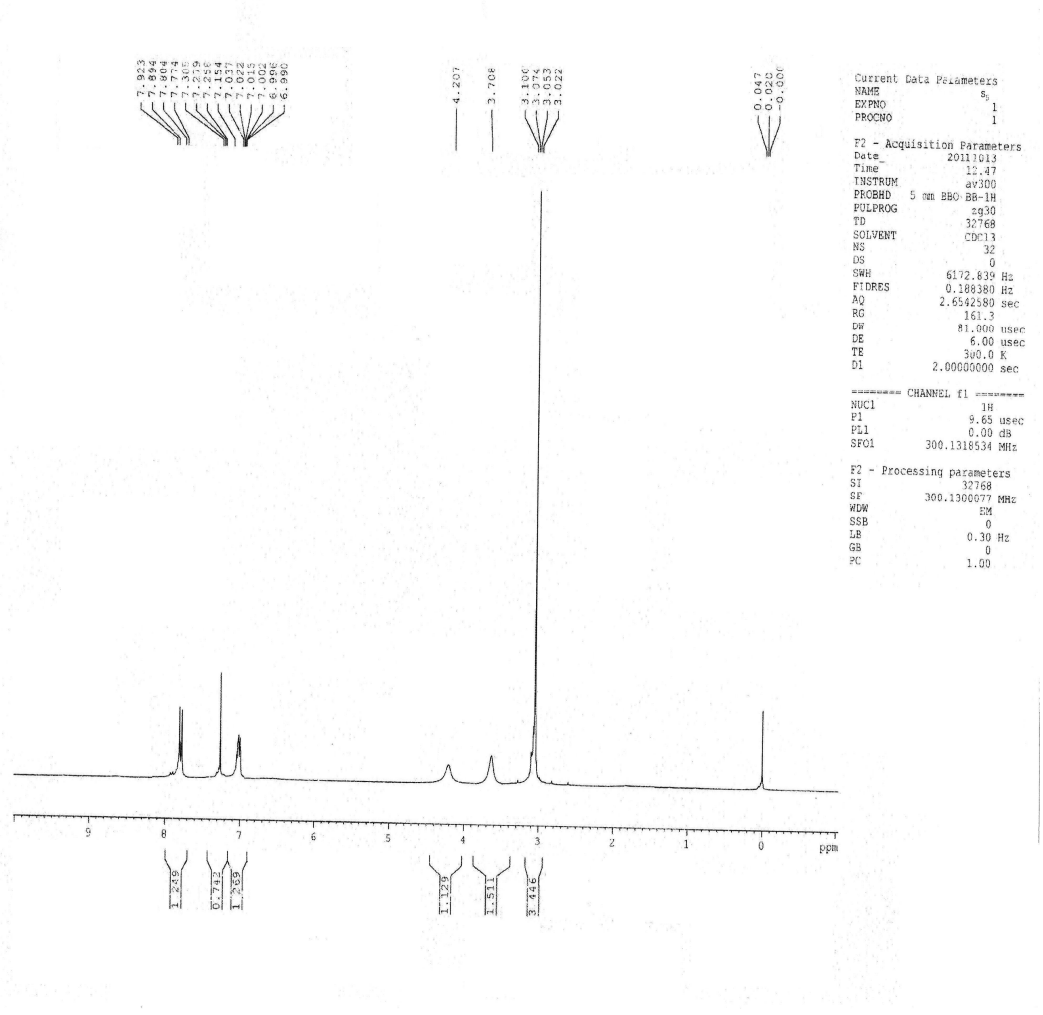
NMR Values

6.990-7.923 (m, 14H, ArH)

4.207 (s, 1H, -N-NH)

3.708 (s, 1H, -NH Ph)

3.058 (s, 2H, -CH_2)



Compound S6

NMR Values

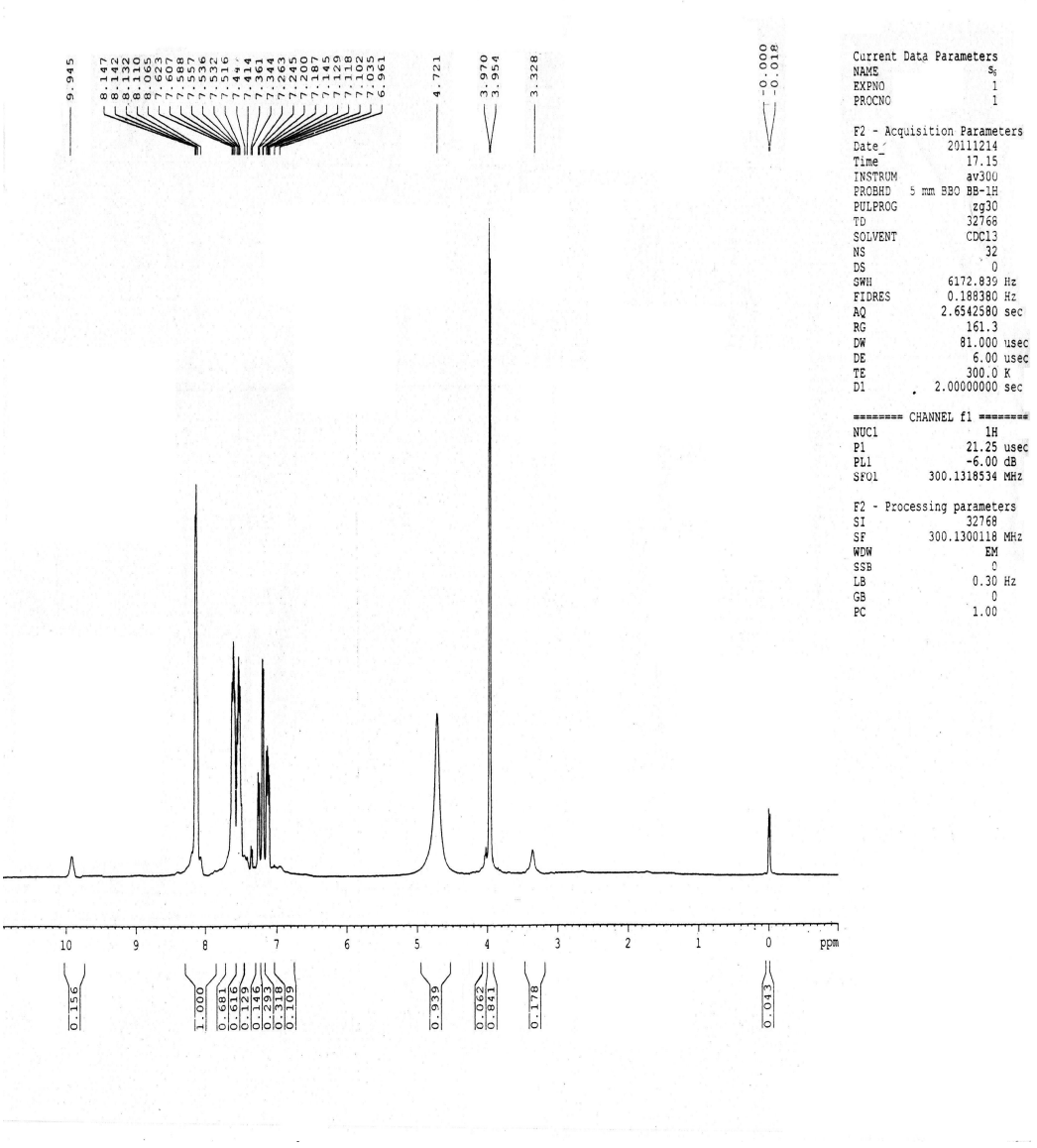
9.945 (s, 1H, -COOH)

6.961-8.147 (m, 13H, ArH)

4.721 (s, 1H, -N-NH)

3.328 (s, 1H, -NH Ph)

3.970 (s, 2H, $-CH_2$)



Compound S7

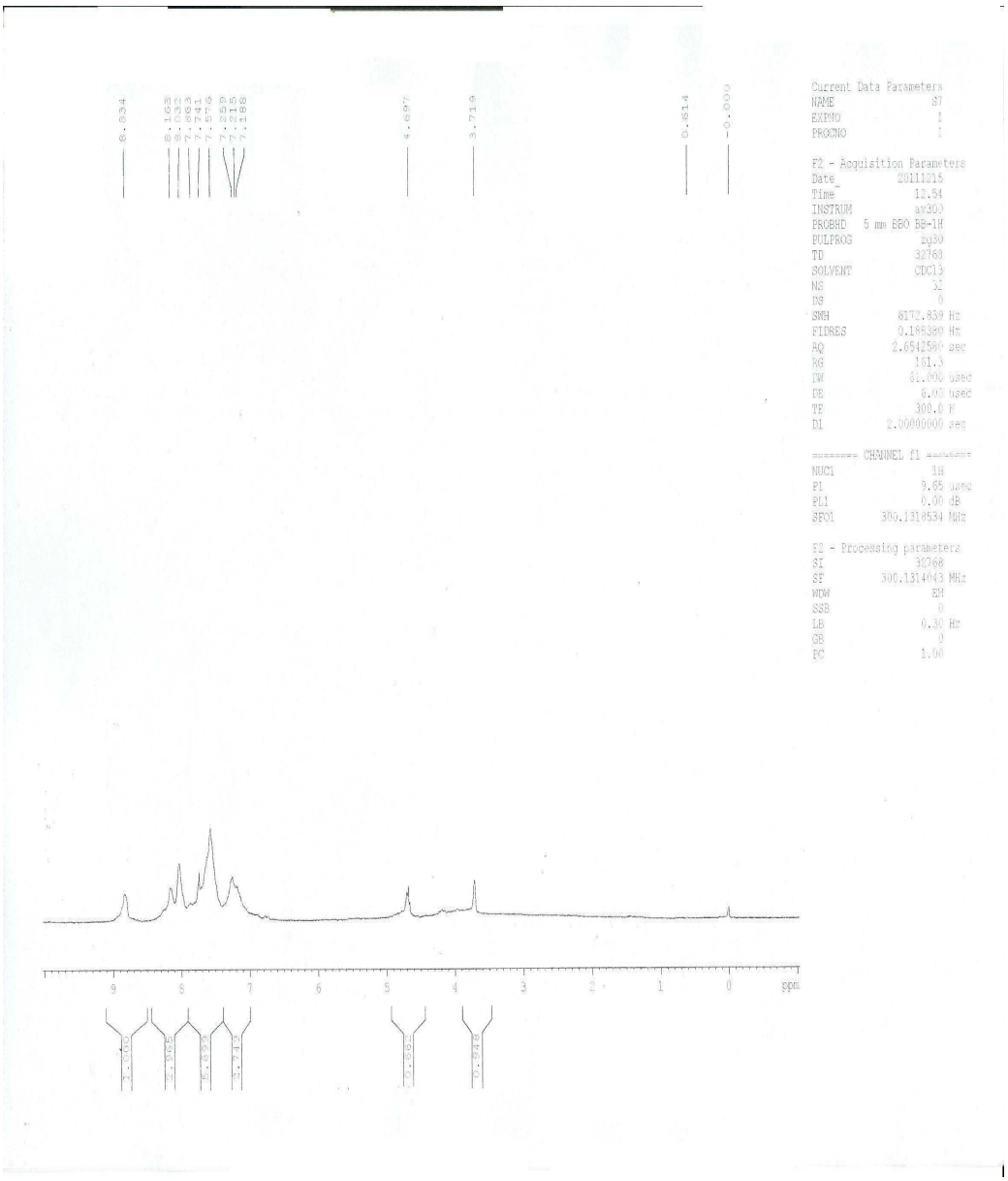
NMR Values

8.834 (s, 1H, -CH of benzimidazole)

7.188-8.163 (m, 13H, ArH)

4.697 (s, 1H, -NH)

3.719 (s, 2H, $-CH_2$)



Compound S8

NMR Values

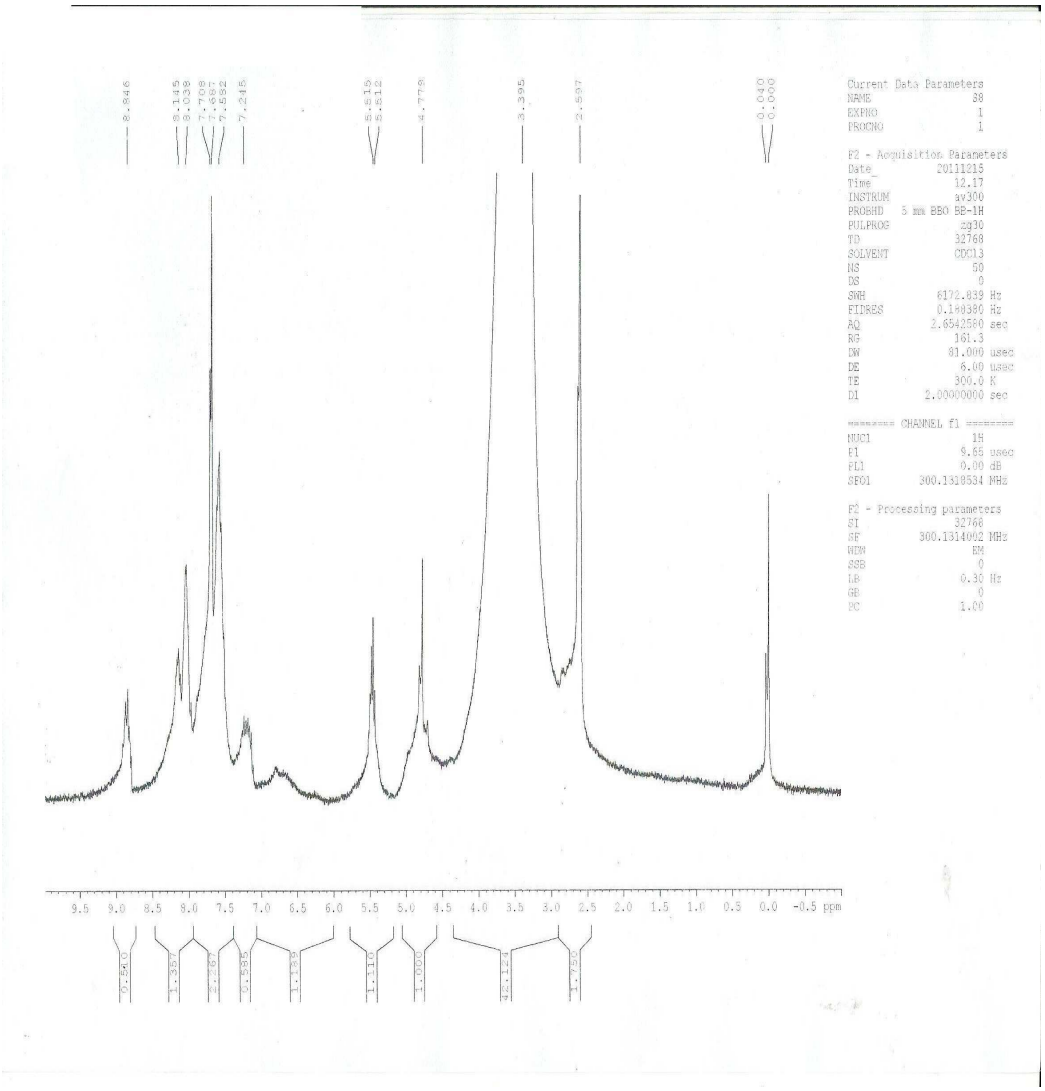
2.597 (s, 2H, CH_2)

3.395 (s, 1H, NH-Ph)

4.779 (s, 1H, -N-NH)

5.512 (s, 2H, SO_2NH_2)

7.245-8.846 (m, 13H, ArH)



Compound S9

NMR Values

6.955-7.527 (m, 9H, ArH)

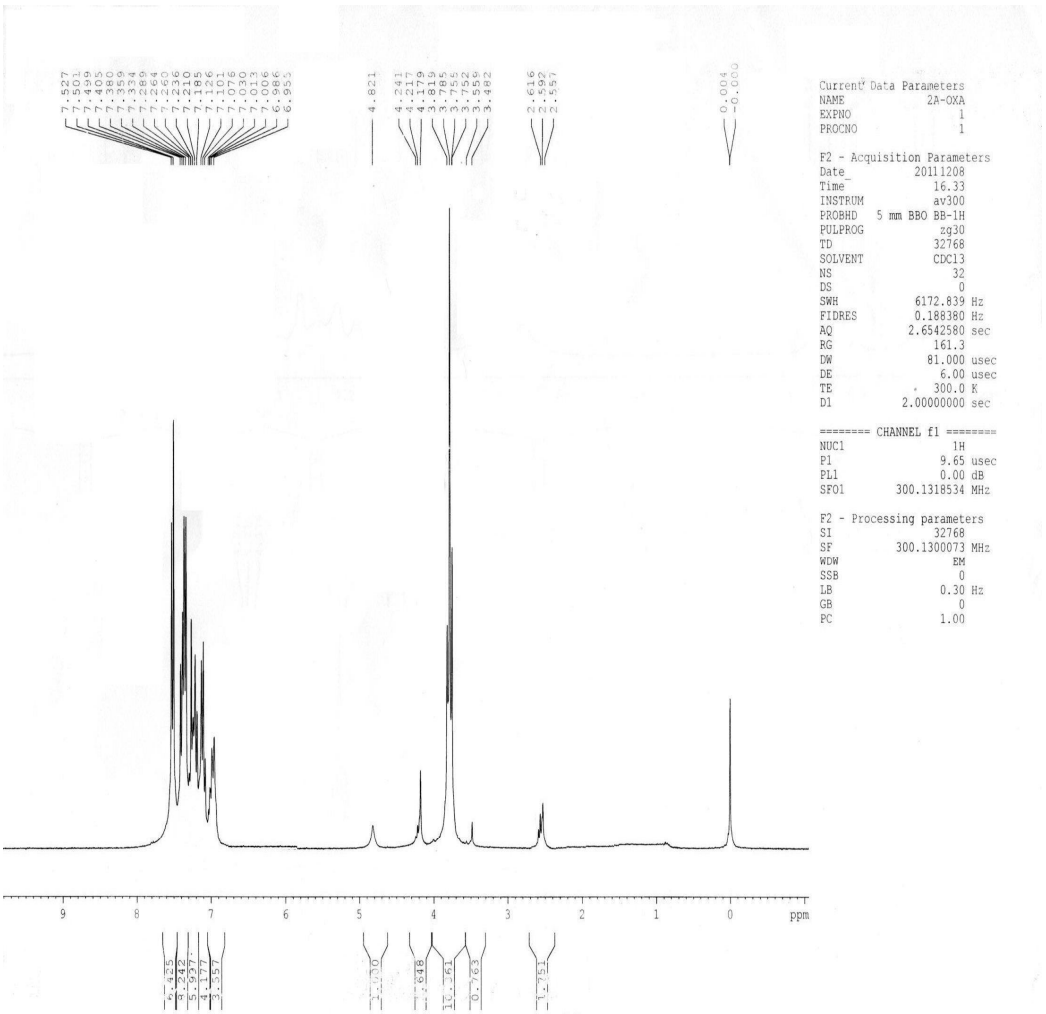
4.821 (s, 1H, -OH)

3.785 (s, 1H, -NH)

3.482 (s, 2H, -CH_2)

2.616-2.557 (t, 2H, N-$\underline{CH_2}$)

4.241-4.179 (t, 2H, $\underline{CH_2}$-OH)



Compound S10

NMR Values

6.671-7.574 (m, 9H, ArH)

4.871 (s, 1H, -NH)

3.714 (s, 2H, -CH_2)

3.386 (s, 2H, (2,6) CH_2 of morpholine)

2.563 (s, 2H (3,5) CH_2 of morpholine)

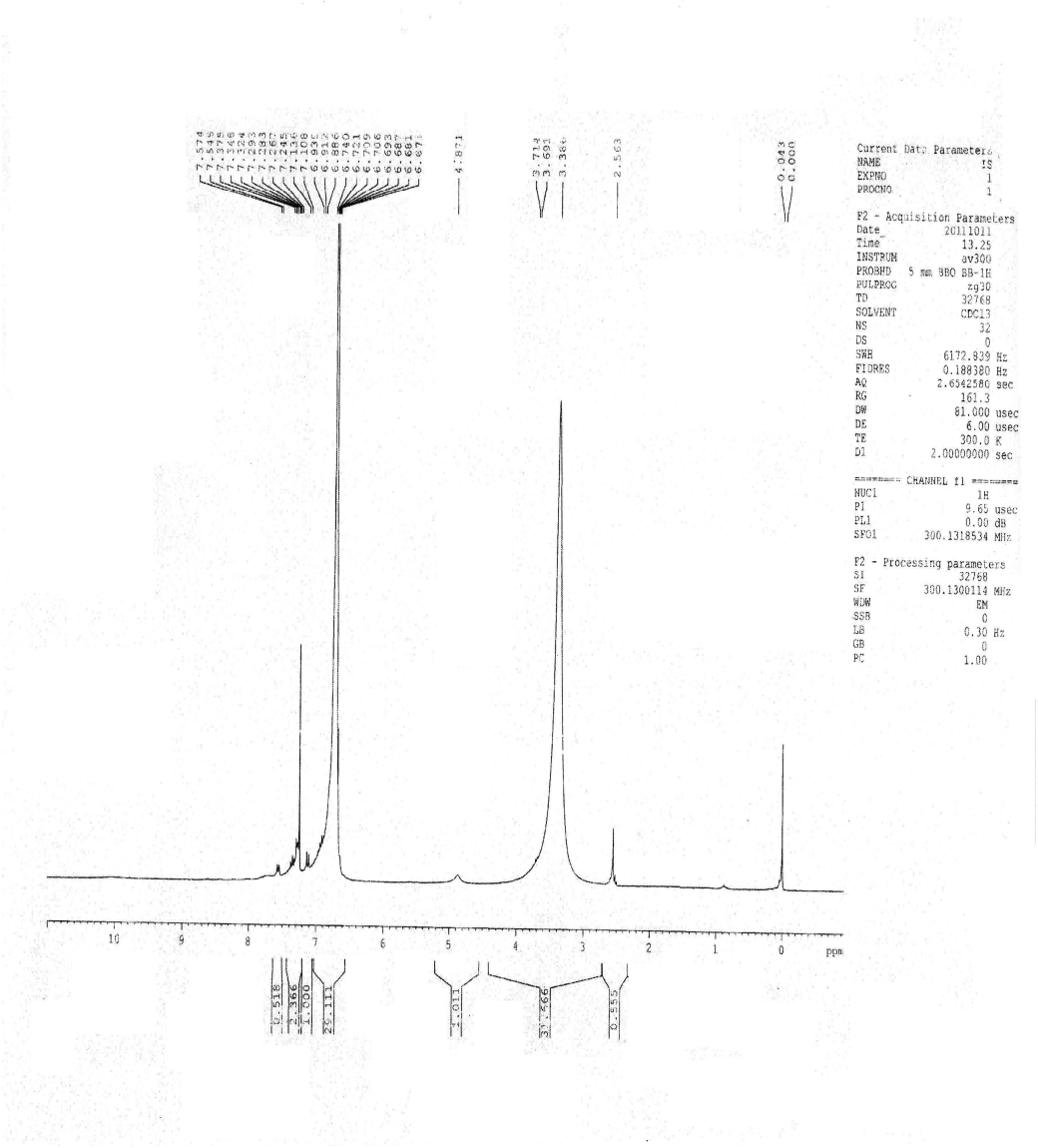
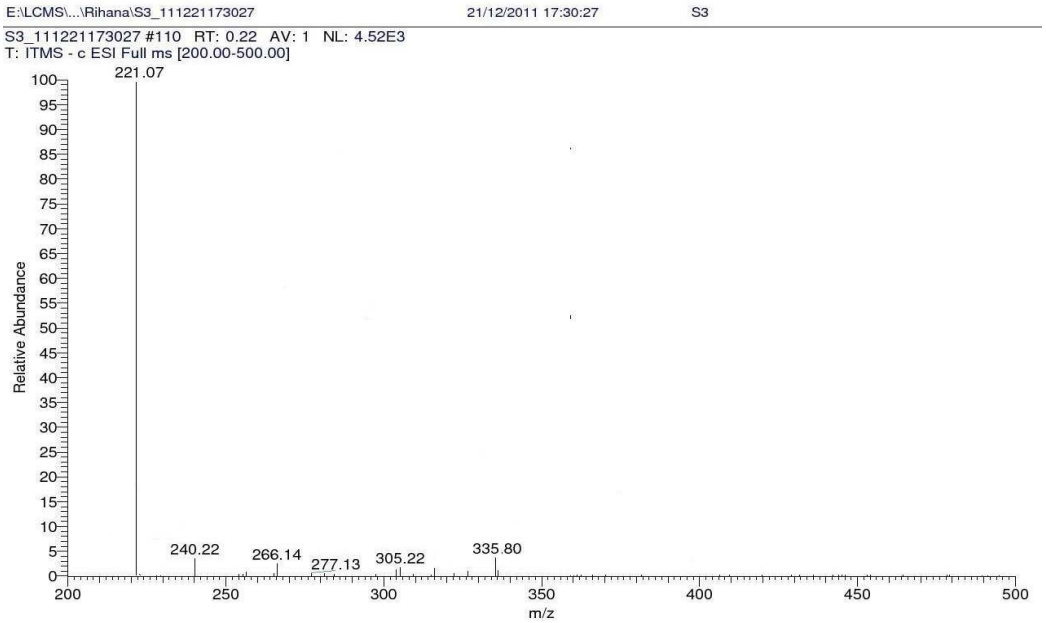


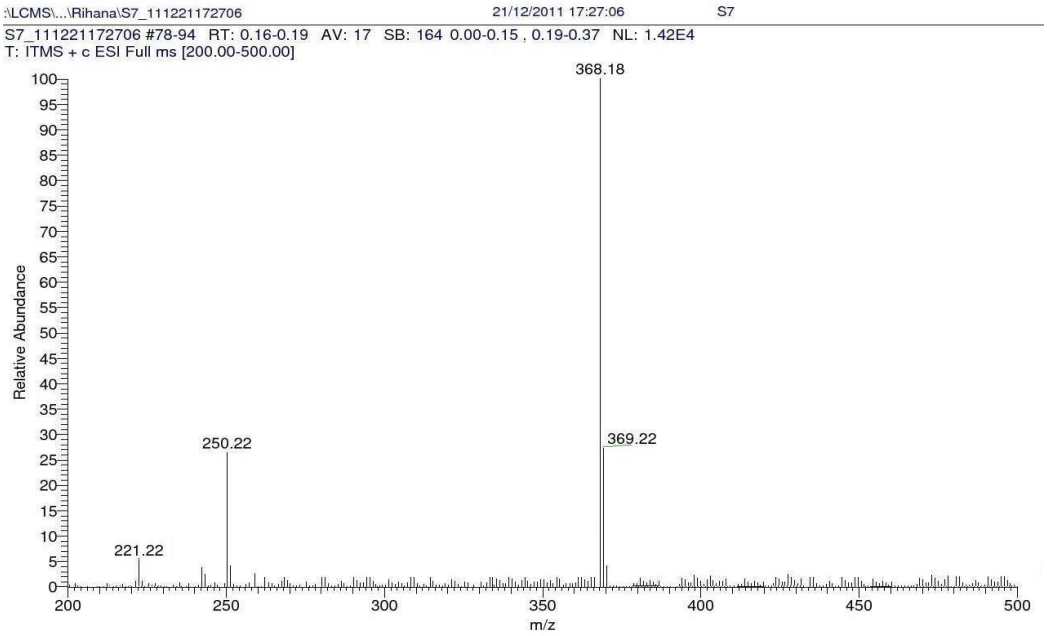
Table-5
Mass Spectra

S. No	Compound Code	Peak Value
1	S3	335.80 (335)
2	S7	368.18 (367)
3	S9	354.32(354)
4	S10	336.32 (336)

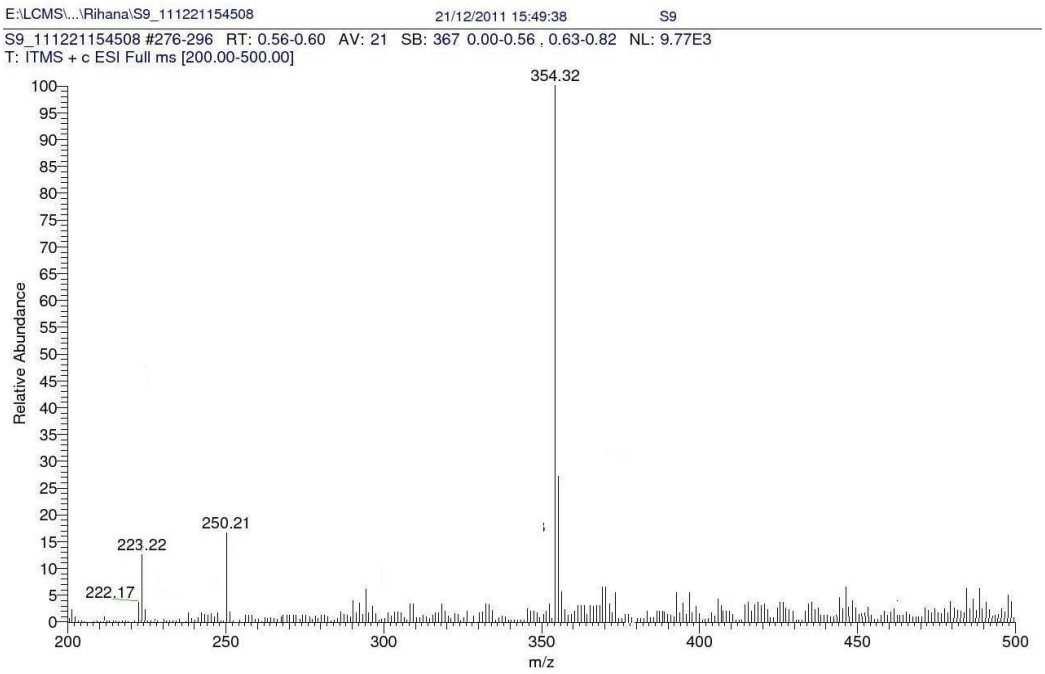
Compound S3



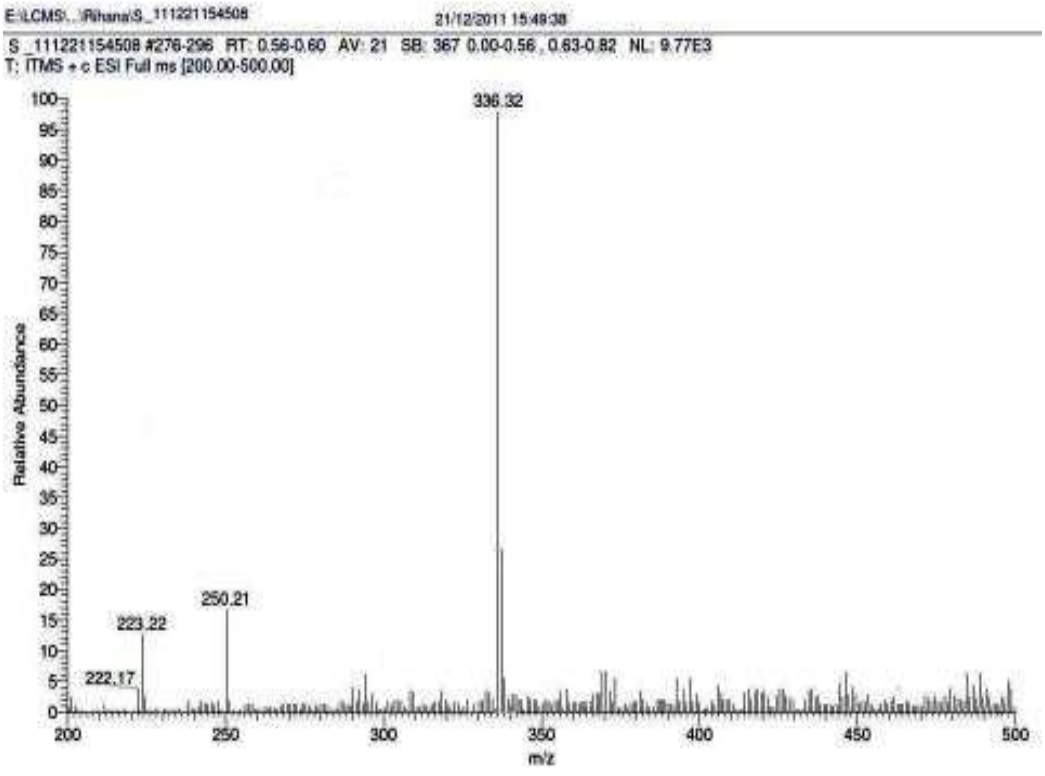
Compound S7



Compound 9



Compound S10



Chapter-VI

Chapter VI

Docking results

INTRODUCTION:

***IN-SILICO* DRUG DESIGN**

Drug discovery and development is an essential, intense, lengthy and an interdisciplinary endeavor. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development.

Traditionally drugs were discovered by synthesizing compounds in a time consuming multi-step processes against battery *in-vivo* biological screens and further investigating the promising candidates for the pharmacokinetic properties, metabolism and potential toxicity. Such a development processes has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the processing of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, *de novo* design *in vitro, in silico* ADMET screening and structure- based drug design.Computer aided drug design is an interdisciplinary of bioinformatics, medicine and biophysics. Bioinformatics and computational methods recently were used to design new drug candidates that could potentially bind with target proteins, thus producing drug

molecules for many disease. They also promise to speedup drug research by predicting potential effectiveness of designed compounds prior to experimental studies and preclinical trials.

In-silico methods can help in identifying the drug targets via bioinformatics tools. They can also be used to analyze the target structure for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their biding affinities, further optimize the molecules to improve binding characteristics. The use of computers and computational methods permeates all aspects of drug discovery today which is essential core of structure-based drug design. The use of *in-silico* drug design techniques increases the chance of success in many stages of the drug discovery process, from the identification of novel targets and elucidation of their function to the discovery and development of lead compounds with desired properties. Computational tools provide the advantage of delivering the new drug candidates more quickly and at lower cost.

RATIONAL DRUG DESIGN

In-silico techniques save great amounts of time and money in R&D projects. A good modeling support is often what makes the difference between a successful drug design project and one that fails. With a strong background in the fields of molecular modeling, molecular biology and computational chemistry, we are able to offer full *in-silico* support for projects of drug design, protein engineering and intermolecular recognition. The possibility of developing software to tailor the *in-silico* approach to different problems is what makes us unique.

TECHNIQUES

- **Molecular Docking and Virtual Screening**: Docking studies are computational techniques for the exploration of the possible binding modes of a substrate to a given receptor, enzyme or other binding site. Docking is the process by which two molecules fit together in 3D space. Docking studies may help to increase ligand specificity; and also better therapeutic index can be achieved if the drug produces undesirable side effects due to its binding with another site, the affinity for that competing site can be diminished. Different types of docking include- flexible protein-ligand docking, flexible protein-protein docking and hydrophobic docking. Docking may play an important role in the QSAR studies and homology modeling very useful in structure based drug design. Various docking programs are available DOCK, FLOG, ADAM, and UGIN.
- **Molecular Dynamics**: The prediction of the evolution of molecular systems over time, the study of protein conformation, protein-protein interactions, the simulation of biological membranes.
- **Quantum Mechanics**: The study of chemical reactions, the effects of substitutions on electronic properties and reactivity of molecules.
- **QSAR**: Quantitative structure-activity relationship. The ability of predicting biological properties of molecules without even the need of knowing their target.
- **Homology Modelling**: Predicting the structures of proteins that has not been yet crystallized.

DOCKING STUDIES:

The ability to propose reasonable binding modes of a designed structure to a known receptor site called docking studies, which is crucial to the success of structure based design. One approach is to dock or position ligand or receptor molecules together in many different possible ways and then scores each orientation according to an evaluation function of some kind. These studies can predict binding confirmations and affinities of millions of molecules without the need of a single synthetic step. These rational drug design methods accelerate the process by speeding up the discovery of new chemical substances that may become a new drug.

DRUG-LIKENESS AND LEAD-LIKENESS

Christopher A. Lipinski defined the Drug likeness as the compounds those have sufficiently acceptable absorption, distribution, metabolism and elimination properties to get successful entry in to human Phase 1 clinical trials. For the drug development, drug properties are important prominent component. A chemically synthesized compound library can contain many non-drug-like compounds. Therefore, recent technologies helped to develop recognized drug-like compounds from a diverse compound library. These drug-like measuring and filtering technologies have partly solved the screening problems. However, they have not been good enough to completely solve these problems. It has been observed that many drug-like compounds, which should be potential candidates; do not come up as hits when they are screened against biological targets. Drug-likeness is the descriptors of all important pharmacological properties such as potency, selectivity toward receptor, absorption, distribution, metabolism and toxicity. In the past, these parameters were

optimized sequentially. Now, it is mandatory that these parameters should be optimized simultaneously. Properties that have been associated with oral drug-likeness include:

- Oral bioavailability
- Appropriate toxicity to pass phase I clinical trials.
- Aqueous solubility
- Synthetics accessibility
- Pharmacokinetic viability
- Blood-brain barrier permeability.

Lipophilicity is a key property for pharmacological activity in drug discovery and used to estimate the permeability of a drug molecule in the cell membrane. It is measured as logP value that distribution coefficient of compounds between n-octanol and water.

When logP value is very low or very high, the permeability of drug components get dropped due to the inability of weakly lipophilic compounds to penetrate the lipid portion of the membrane and the excessive partitioning of strongly lipophilic compounds into the lipid portion of the membrane and their subsequent inability to pass through the aqueous portion of the membrane.

Lipinski's rule helps to predict the poor absorption and permeability of potential drug candidates. It will occur if,

- A molecular weight less than 500.
- An octanol-water partition coefficient log P of less than 5.
- Molar refractivity not more than 150

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).

TOOLS AND MATERIALS USED

Auto Dock is an automated docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. Auto Dock actually consists of two main programs: one performs the docking of the ligand to a set of grids describing the target protein; and the other Auto Grid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can be visualized. A graphical user interface called Auto Dock Tools or ADT was utilized to generate grids, calculate dock score and evaluate the conformers.

Accelrays Discovery Studio:

Accelrays Discovery Studio is a molecular graphics program intended for the structural visualization of proteins, nucleic acids and small biomolecules. The program reads in molecular coordinate files and interactively displays the molecule on the screen in variety of representations and color schemes.

Computed atlas of surface topography of proteins (CASTp):

Binding sites and active sites of proteins and DNAs are often associated with structural pockets and cavities. castP server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each

pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, area of the openings, and circumference of mouth lips, in both SA and MS surfaces for each pocket.

Selection of target:

Target for anti-tumor drugs:

This work deals with synthesis of new inhibitors of cyclin/CDK complexes.Also, these compounds are potent inhibitors of human cellular proliferation. They are useful in treating a disorder mediated by elevated levels of cell proliferation in a mammal compared to a healthy one by administering an effective dose. In the treatment of proliferative diseases the interruption of the cell cycle is one approach.

The phases of the cell cycle are driven by cyclin-dependent kinases. Upon complexation with its activating proteins, cyclin E or cyclin A, cycline-dependent kinase2 (CDK2) modulates the activity of many cellular substrates via phosphorylation on Ser and/or Thr residues. In complex with cyclin E, cycline-dependent kinase2 (CDK2) plays a paramount role during the G1/S transition of the cell cycle while in complex with cyclin A, it facilitates the progression of the S phase of the cell cycle. Recent evidence also suggests that CDK2 may have a crucial role in the G2 phase of the cell cycle. The importance of cycline-dependent kinase2 (CDK2) for cell cycle progression has led to an active pursuit of small molecule inhibitors of this enzyme as a possible treatment against cancer and other hyper-proliferative disorders. Our current investigation was based on; first, using a structure-guided strategy based on cycline-dependent kinase2 (CDK2) was as appropriate means to

generate CDK2 inhibitors that might prove useful for the therapy of proliferative disorders.

Moreover, several cores have been reported as potent CDK inhibitors including purines, pyrimidine and quinazolines.

Targets for Non-steroidal anti-inflammatory drugs (NSAIDs):

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of inflammation, especially arthritis. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme prostaglandin endoperoxidase, popularly known as cyclo-oxygenase (COX). It was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated and expressed differently. COX-1 provides cytoprotection in the gastrointestinal tract (GIT), whereas inducible COX-2 selectively mediates inflammatory signals. Since most of the currently available NSAIDs in the market show greater selectivity for COX-1 than COX-2, chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration

Materials and Methods

The structures of human cycline-dependent kinase2 (CDK2), COX-1 and COX-2 receptors were retrieved from Protein Data Bank (PDB). All these molecules as well as the bound ligand of the protein were docked by using the software Auto Dock and the score values are predicted. The protein ligand interactions were also studied. All molecules were drawn using ChemDraw Ultra 8.0 tool and energy minimized using Chem 3D Ultra 8.0 software.

A large number of crystal structures were available for human CDK2 in complex with small ligands which bind deeply within the ATP site, and which interact with the kinase motif, of particular interest for the purine ring system, the CDK2 complex (PDB code: 1H0V), COX-2 (PDB code: 1cx2) and COX-1 (PDB code : 1eqx) is available from the PDB.

Docking Studies using AutoDock:

AUTODOCK:

Automated docking was used to locate the appropriate binding orientations and conformations of various inhibitors into the receptor binding pockets. To perform the task, the powerful genetic algorithm method implemented in the program AutoDock 4.0.1 was employed. Before docking the screened ligands in to the protein active site, the protein was prepared by deleting the substrate cofactor as well as the crystallographically observed water molecules and then protein was defined for generating the grid. Grid maps were generated by AutoGrid program. Each grid was centered at the crystal structure of the corresponding receptors. The grid dimensions were 60 A˚ X 60 A˚ X 60 A˚ with points separated by 0.375A˚. For all ligands, random starting positions, random orientations and torsions were used. During docking, grid parameters were specified for x, y and z axes as 38.808, 30.946 and 42.249 respectively.

Selection of active sites in the receptor using CASTp Software:

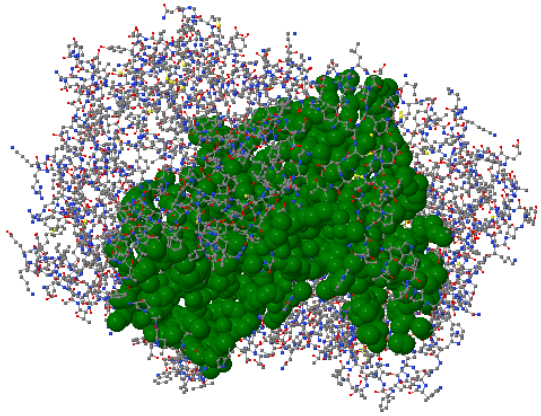


Fig. No 1 Active sites of COX-1

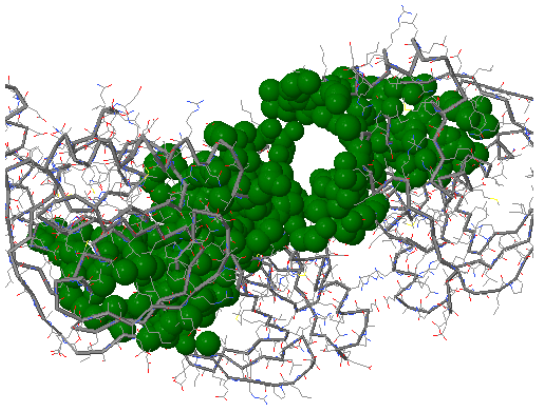


Fig. No 2 Active sites of COX-2

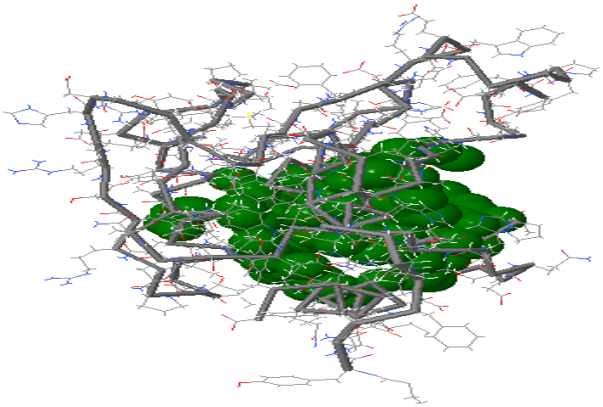
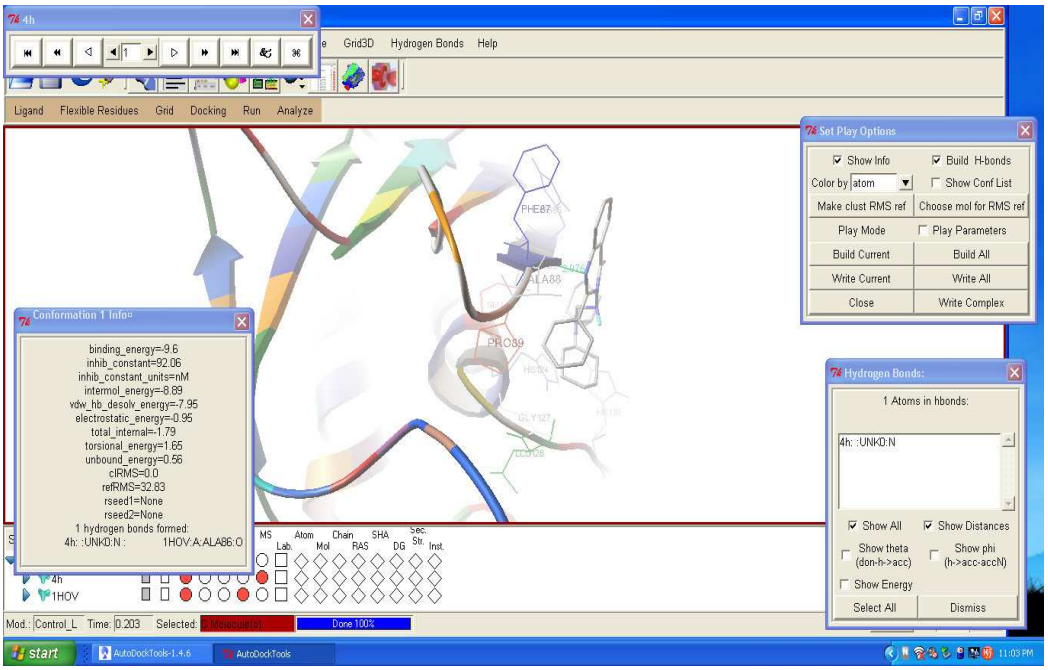


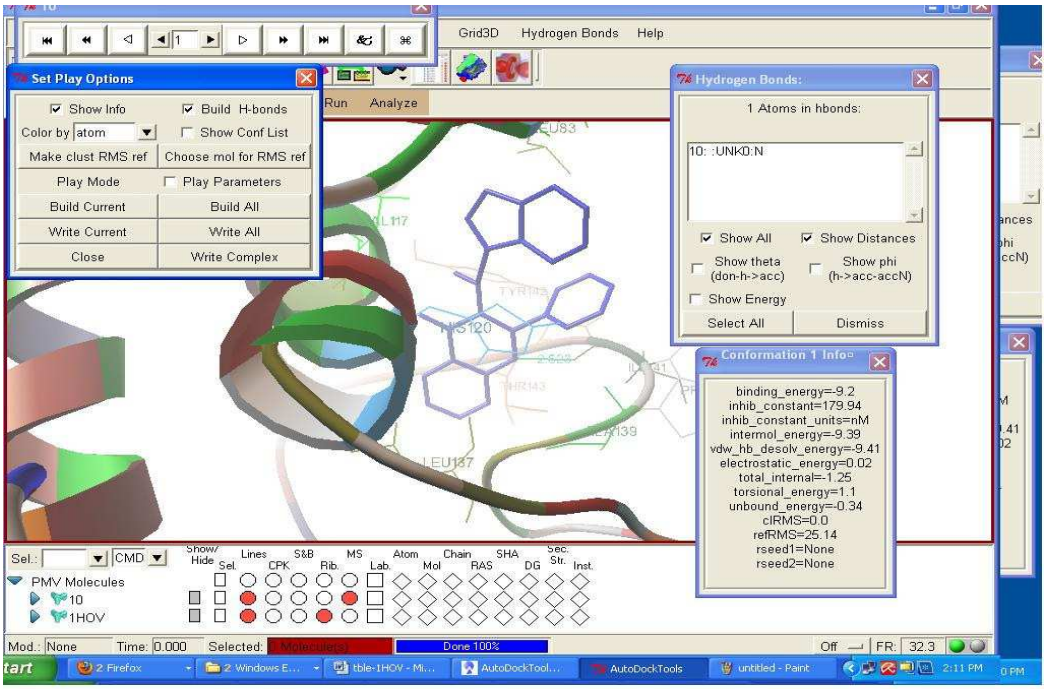
Fig. No 3 Active sites of 1 H0V

Binding mode of compound in the active site of 1- HOV along with interacting amino acids

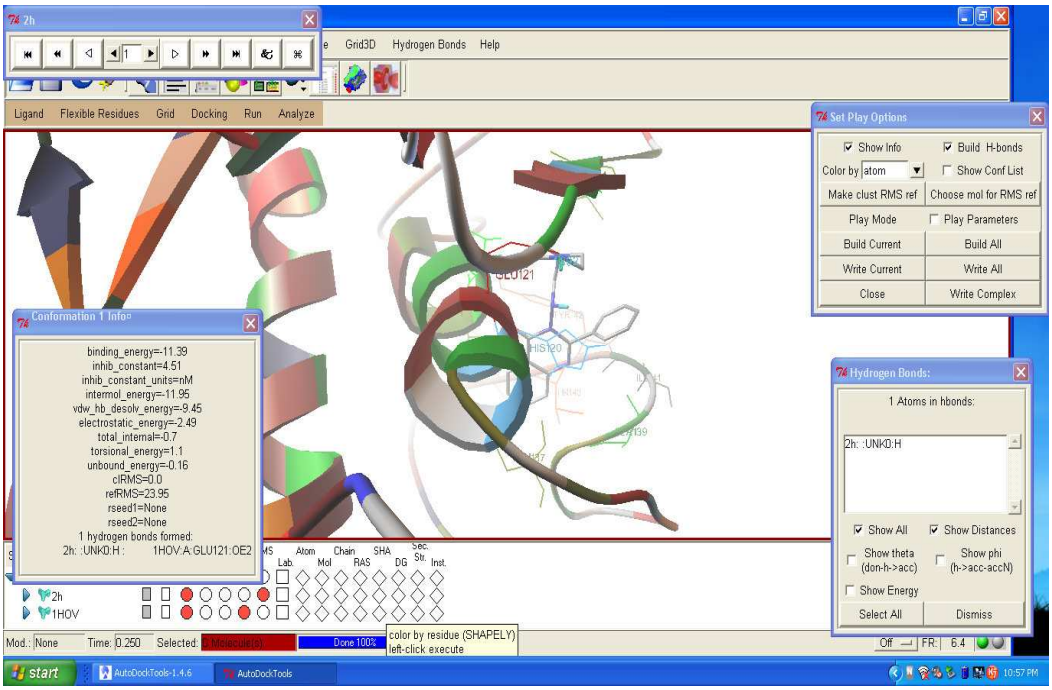
Compound S1



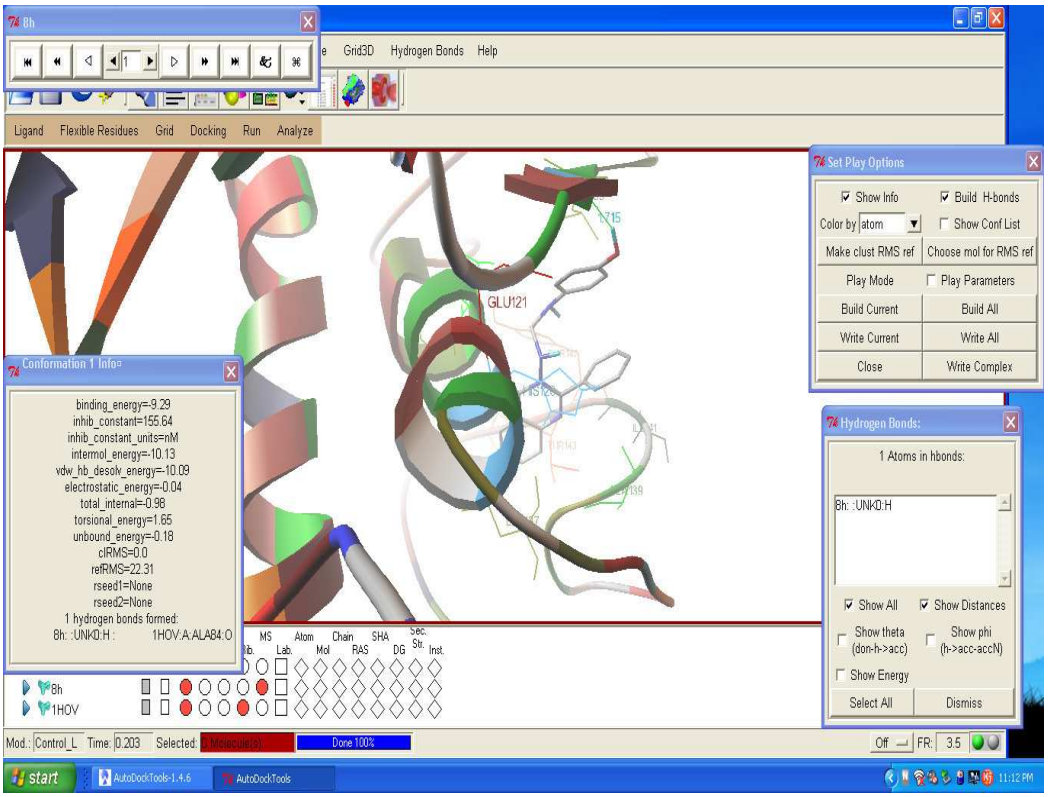
Compound S2



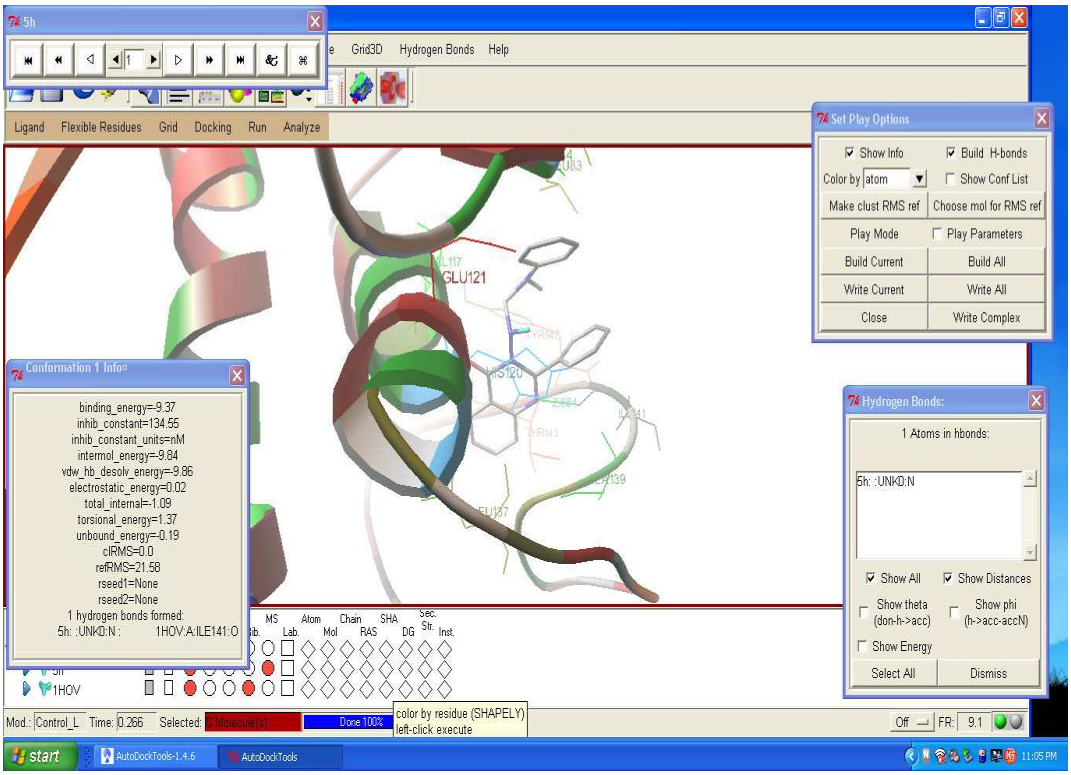
Compound S3



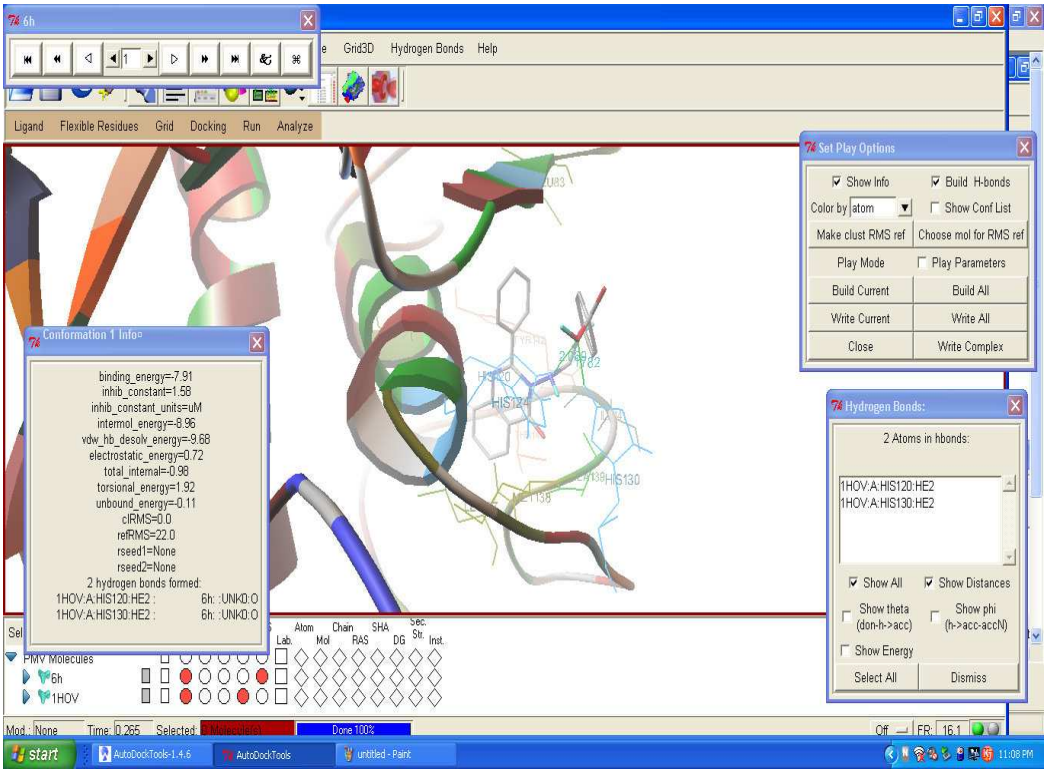
Compound S4



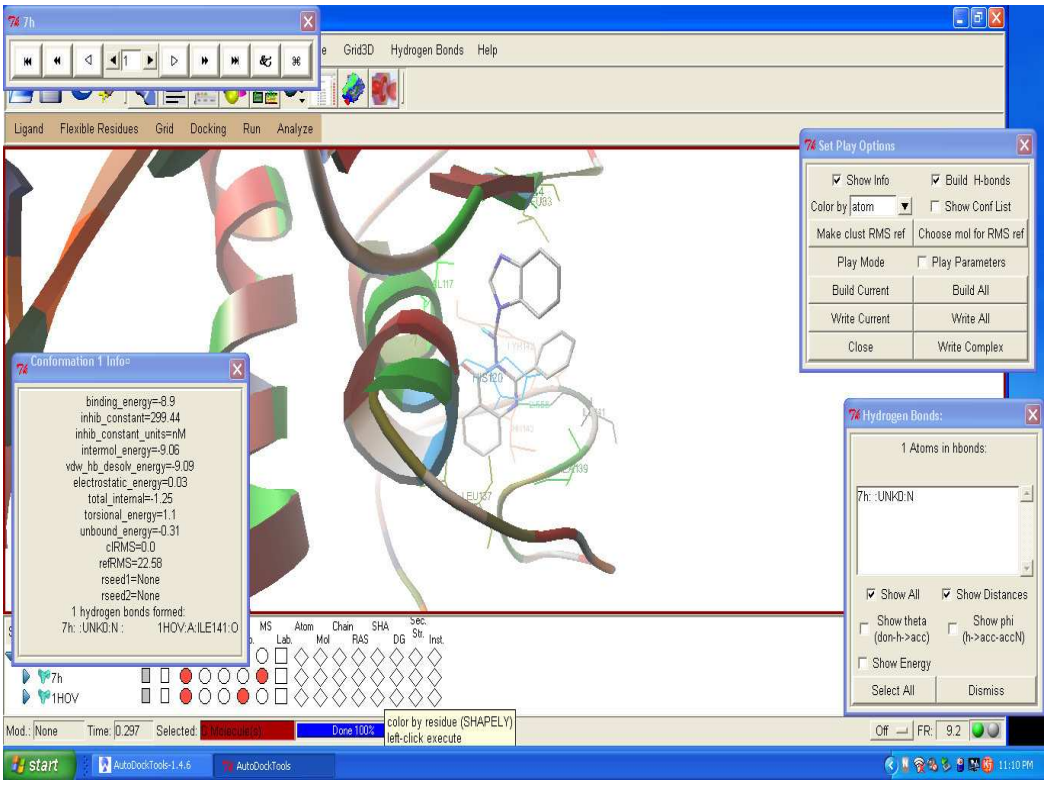
Compound S5



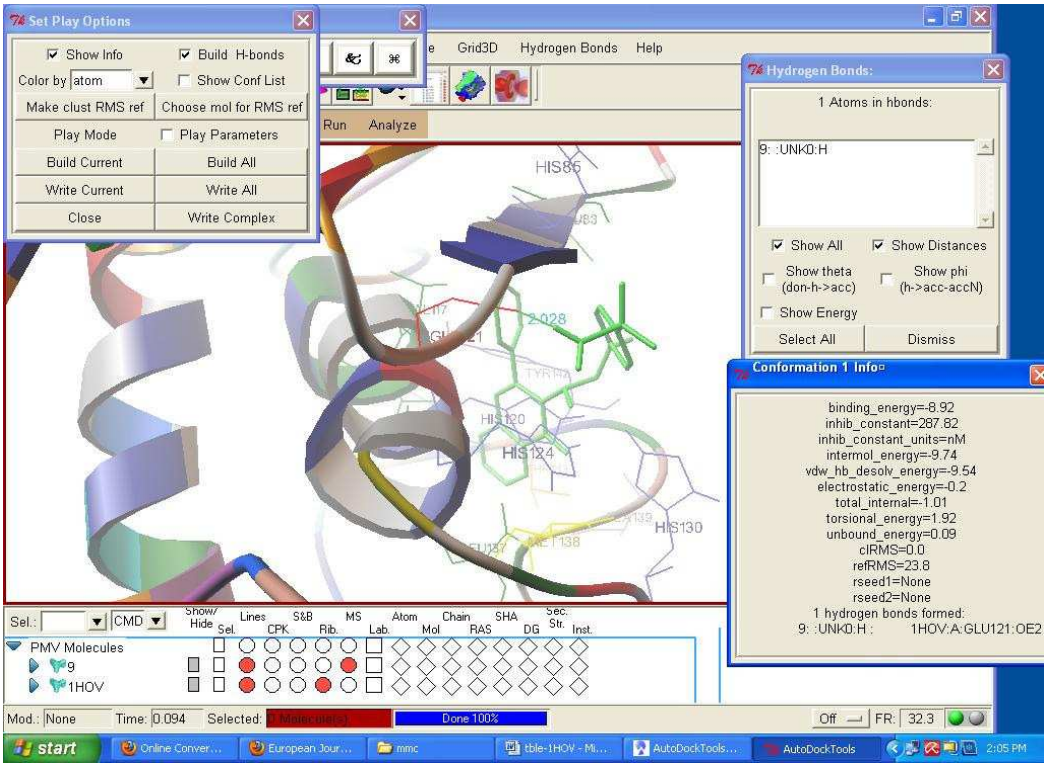
Compound S6



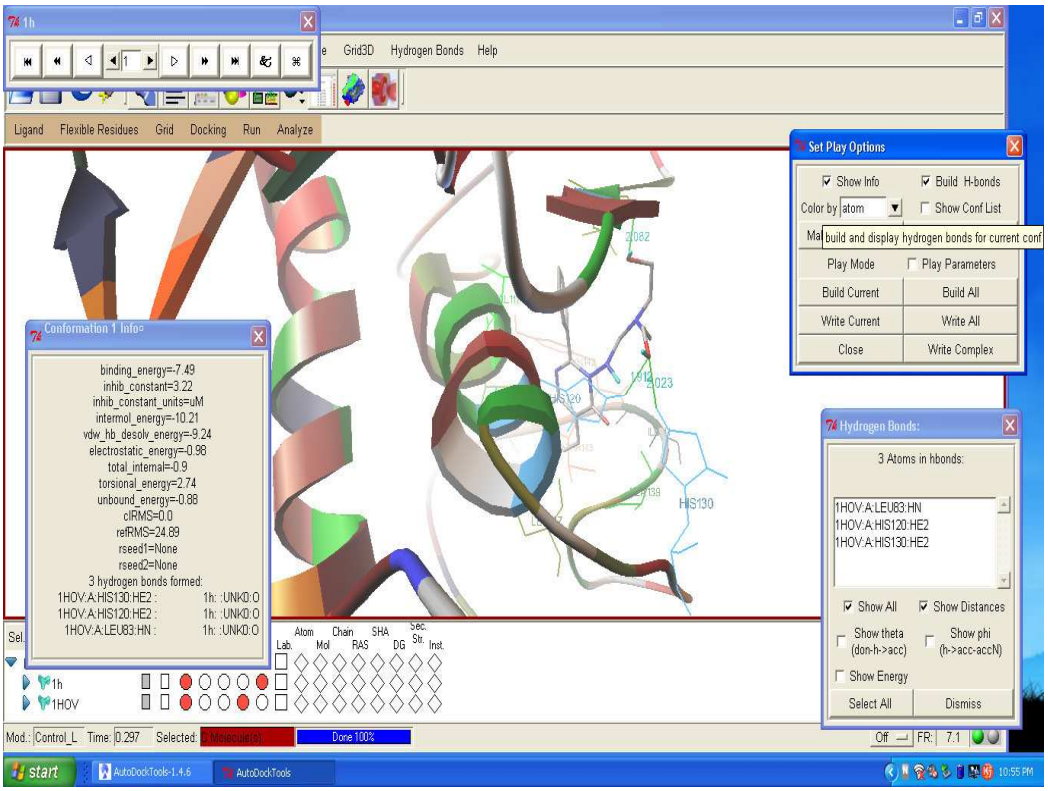
Compound S7



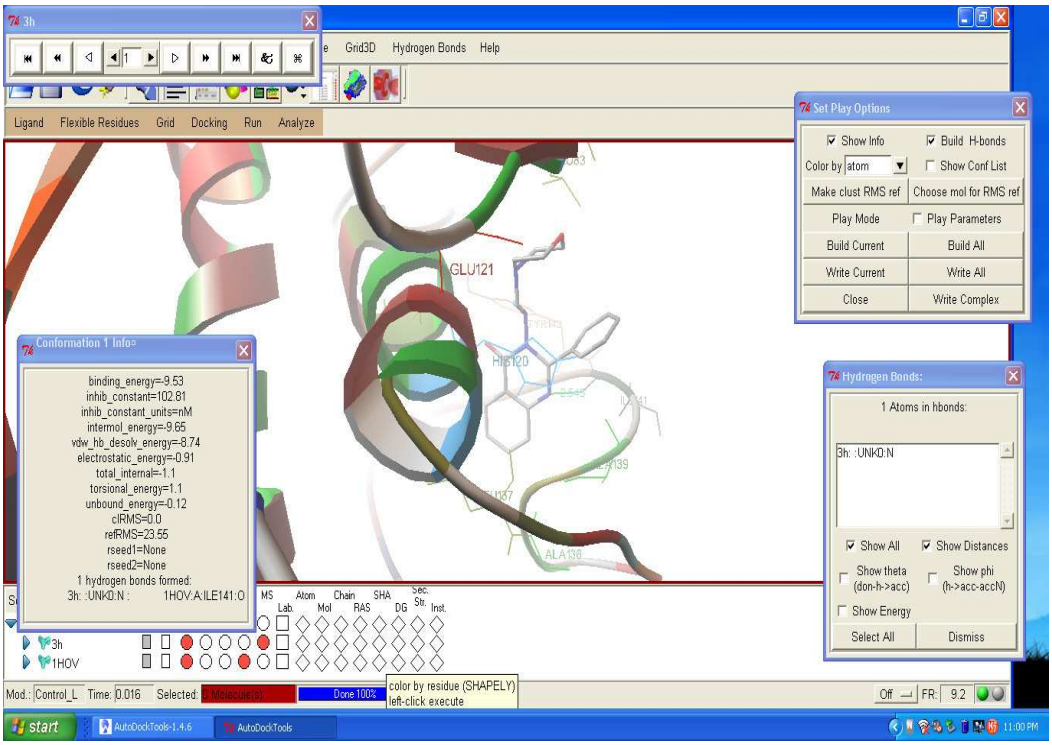
Compound S8



Compound S9

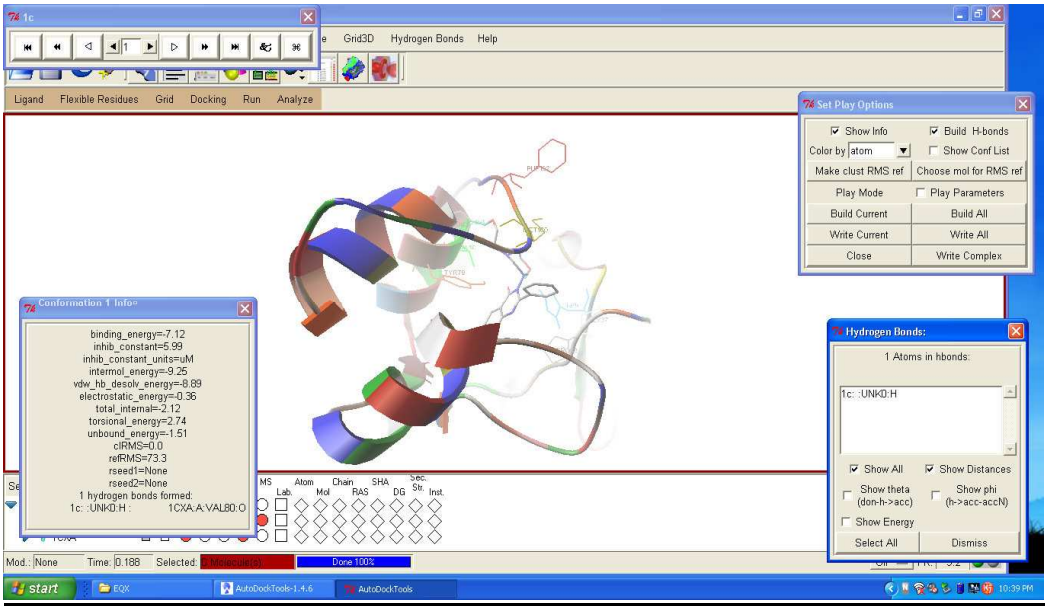


Compound S10

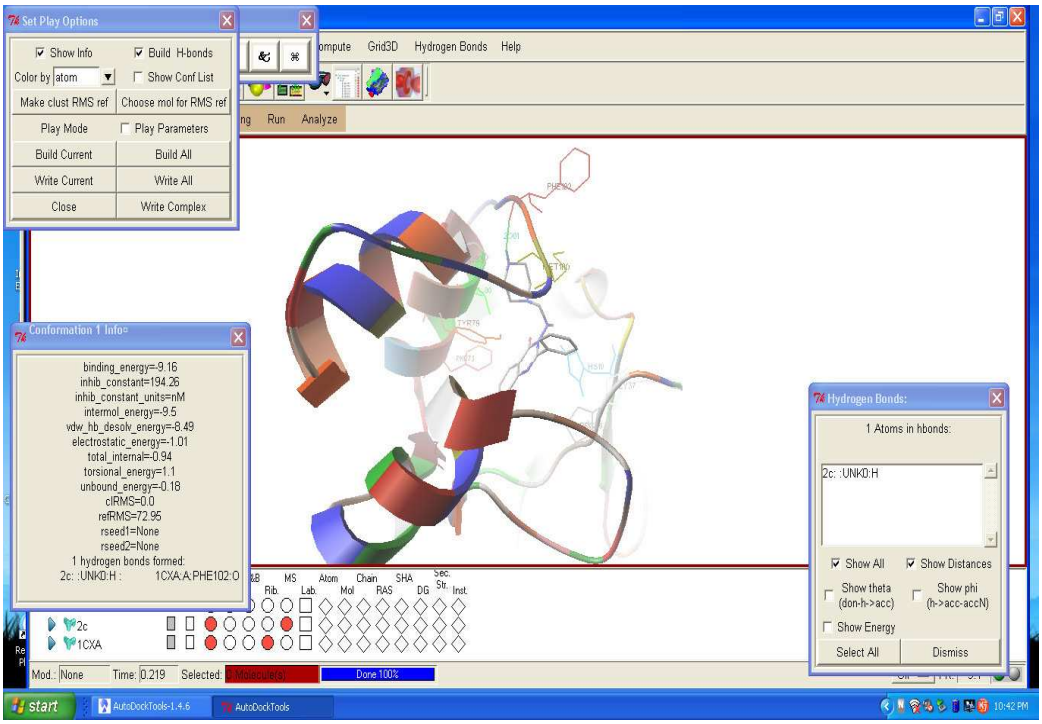


Binding mode of compound in the active site of COX-2 along with interacting amino acids

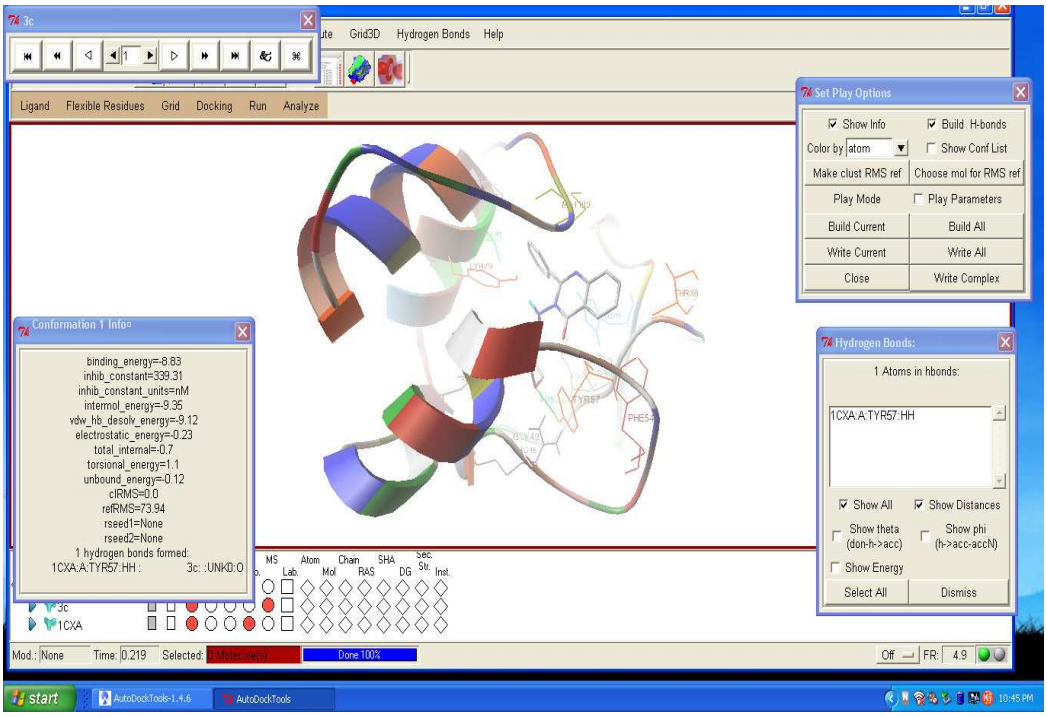
Compound S9



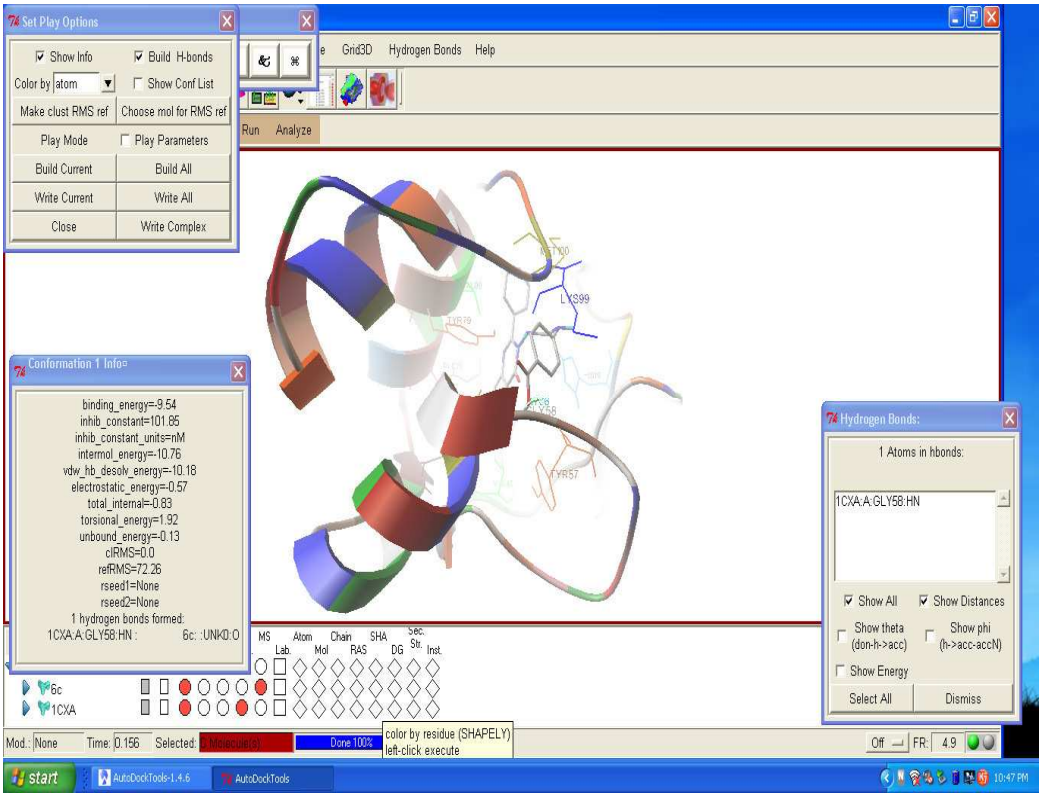
Compound S3



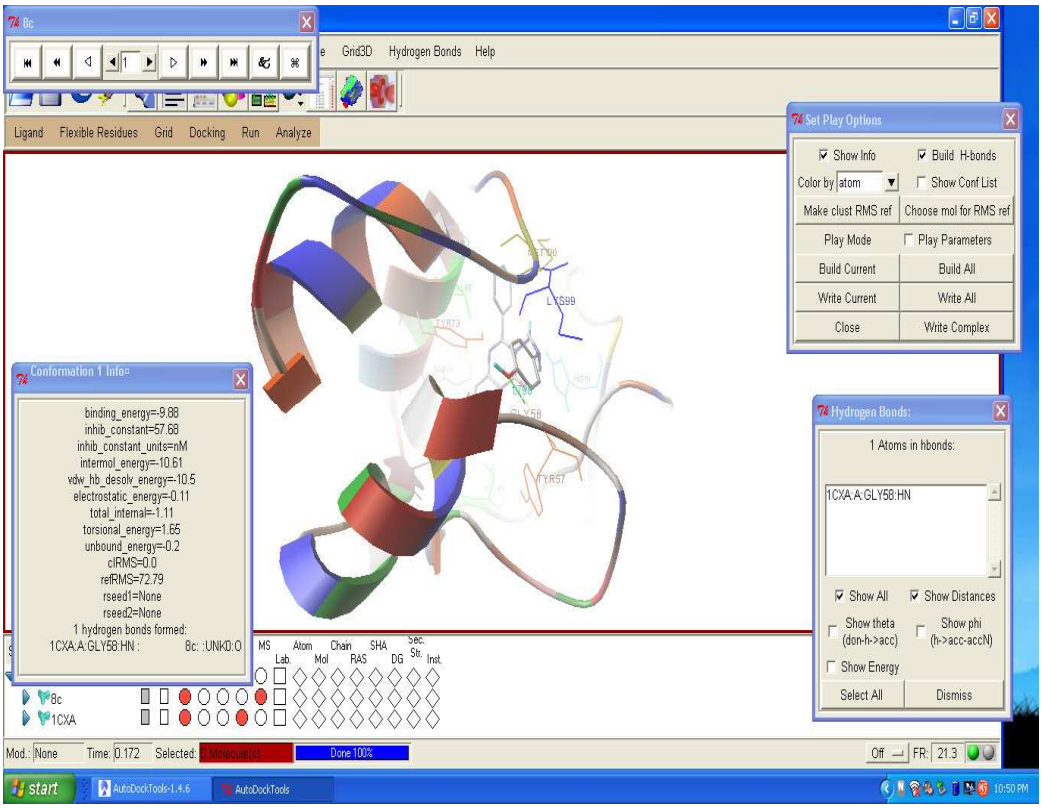
Compound S10



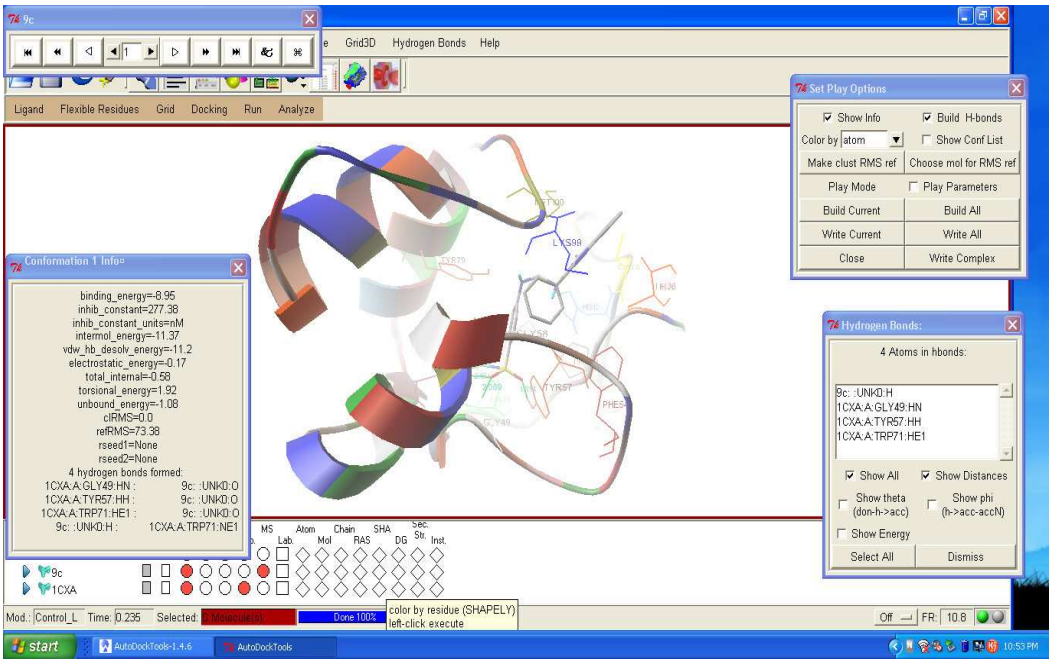
Compound S6



Compound S4



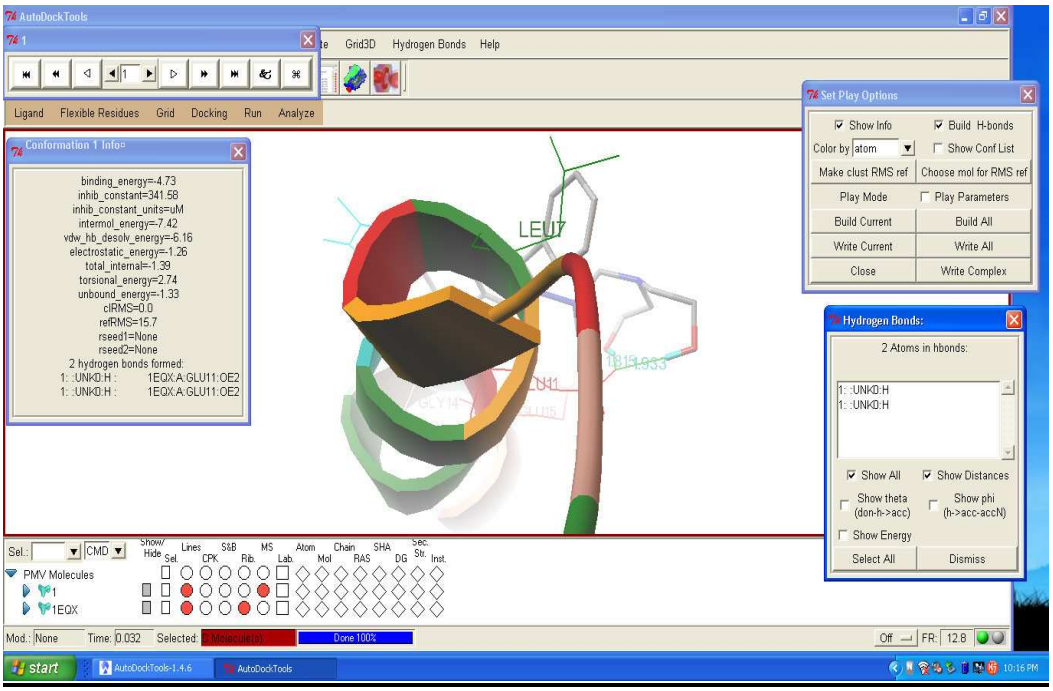
Compound S8



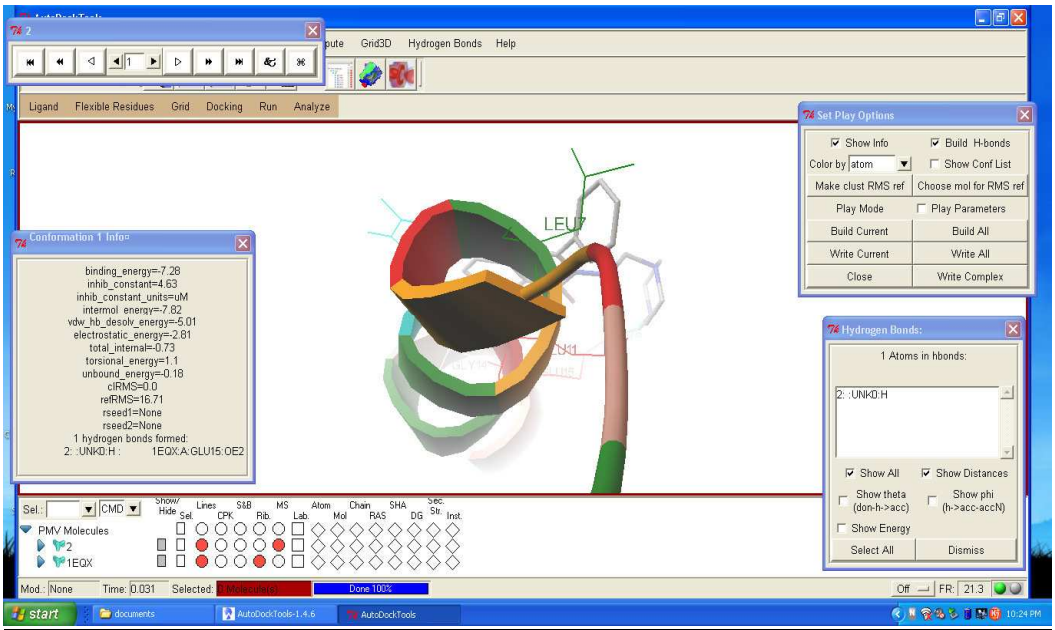
Binding mode of compound in the active site of

COX-1 along with interacting amino acids

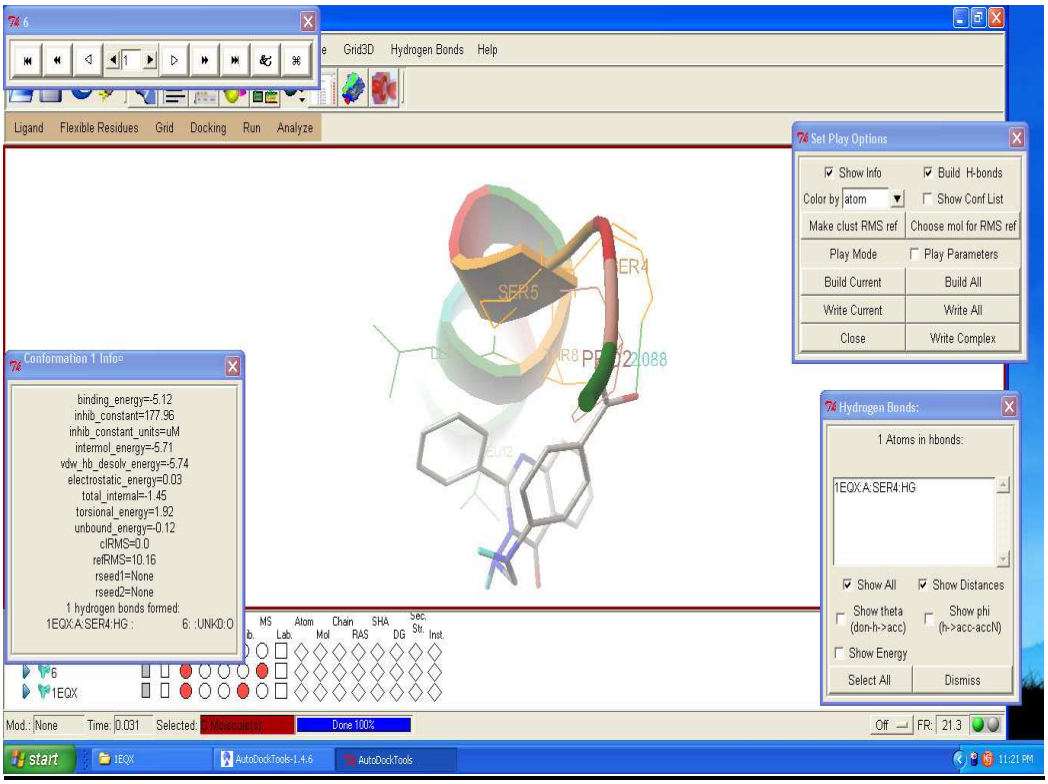
Compound S9



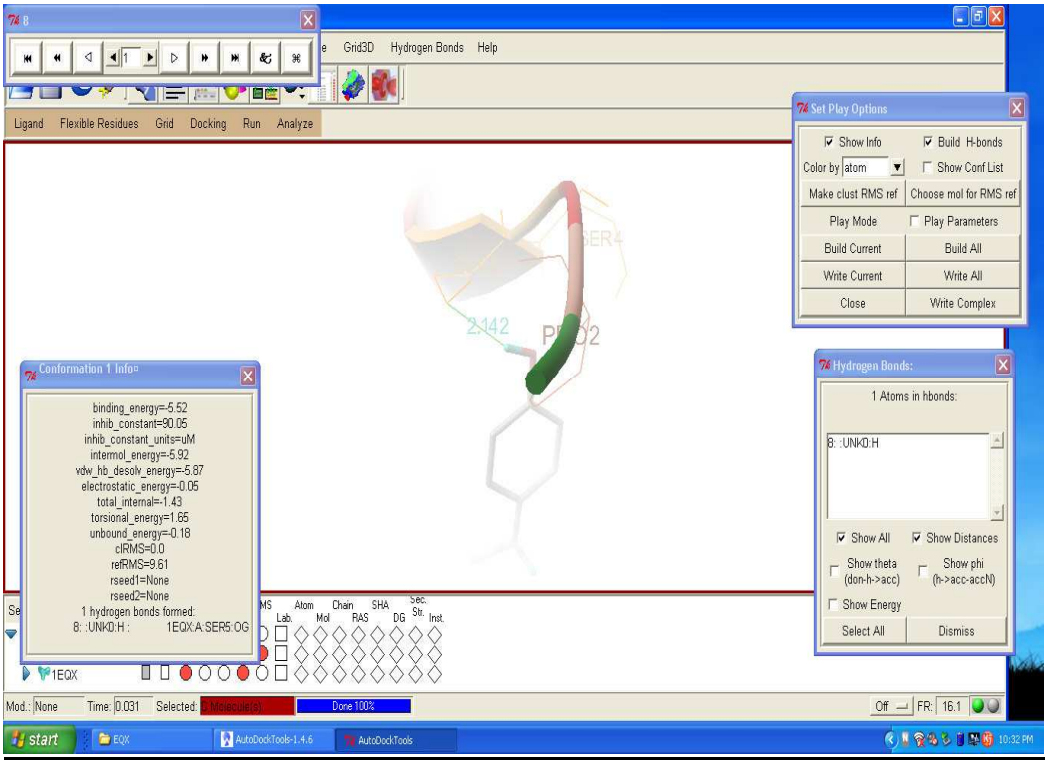
Compound S3



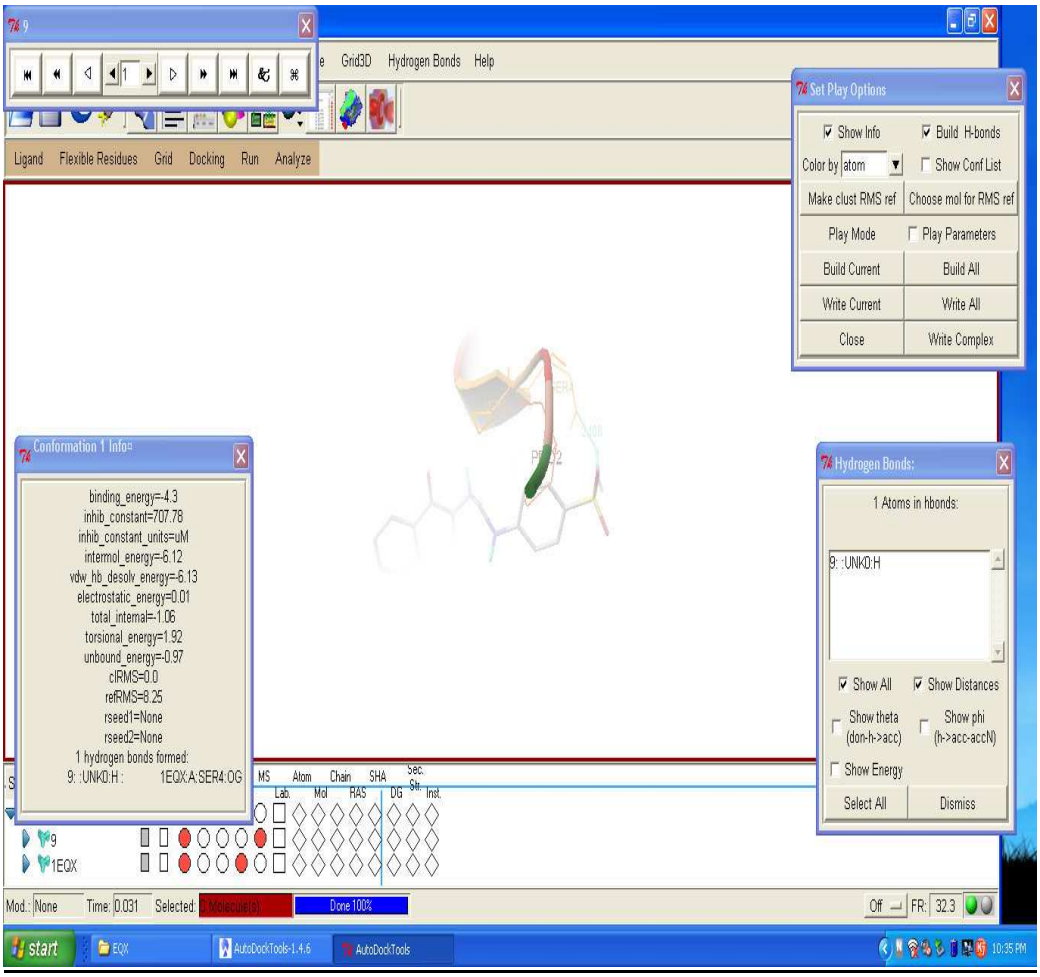
Compound S6



Compound S4



Compound S8


Grid3D
Hydrogen Bonds
Help
Ligand
Flexible Residues
Grid
Docking
Run
Analyze
Set Play Options
Show Info
Build H-bonds
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Make clust RMS ref
Choose mol for RMS ref
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inhib_constant_units=uM
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torsional_energy=1.92
unbound_energy=-0.97
cRMS=0.0
refRMS=8.25
rseed1=None
rseed2=None
1 hydrogen bonds formed:
9: :UNK0:H : 1EQX:A:SER4:OG
Hydrogen Bonds:
1 Atoms in hbonds:
9: :UNK0:H
Show All
Show Distances
Show theta (don-h->acc)
Show phi (h->acc-accN)
Show Energy
Select All
Dismiss
MS
Atom
Chain
SHA
Lab
Mol
RAS
DG
Sec. Str.
Inst.
9
1EQX
Mod.: None
Time: 0.031
Selected: 1 Molecule(s)
Done 100%
Off
FR: 32.3
start
EQX
AutoDockTools-1.4.6
AutoDockTools
10:35 PM

Table: 6, DOCKING STUDIES FOR ANTI-TUMOR ACTIVITY

COMP.	ΔG	INHIBITION CONSTANT	NO. OF H-BONDS	INTERACTING AMINOACID RESIDUES
1(S9)	-7.49	3.22 µm	3	1HOV:A:LEU83:HN 1HOV:A:HIS120:HE2 1HOV:A:HIS130:HE2
2(S3)	-11.39	4.51 nm	1	1HOV:A:GLU121:OE2
3(S10)	-9.53	102.81 nm	1	1HOV: A: ILE141:O
4(S1)	-9.6	92.06 nm	1	1HOV:A:ALA86:O
5(S5)	-9.37	134.55 nm	1	1HOV:A:ILE141:O
6(S6)	-7.91	1.58 µm	2	1HOV:A:HIS120:HE2 1HOV:A:HIS130:HE2
7(S7)	-8.9	299.44 nm	1	1HOV:A:ILE141:O
8(S4)	-9.29	155.64 nm	1	1HOV:A:Glu 121:O
9(S8)	-8.92	287.82	1	1HOV:A:ALA84:O
10(S2)	-9.27	161.94 nm	1	Unknown: N

DOCKING STUDIES FOR ANTI-INFLAMMATORY ACTIVITY:

Table :7 Docked scores of newly designed compounds with COX-2 and COX-1

Comp.	Auto Dock Score (Kcal/mol)		K_i (µM)		No of H-bonds		Interacting amino acid residues	
	COX-2	COX-1	COX-2 nM	COX-1 uM	COX-2	COX-1	COX-2	COX-1
1(S9)	-7.12	-4.73	5.99 uM	341.58	1	2	1CXA;A Val 80:O	1EQX A; Glu 11:O, 1EQX A; Glu 11:O,
2(S3)	-9.16	-7.28	194.26	4.63	1	1	1CXA;A Phe 102:O	1EQX A; Glu 15:O,
3(S10)	-8.83	-5.93	339.31	44.87	1	0	1CXA;A Tyr 57:HH	-
4(S1)	**-10.43**	**-4.76**	**22.15**	**326.53**	**0**	**0**	**--**	**--**
5(S5)	-9.58	-5.53	94.32	88.92		0		-
6(S6)	-9.54	-5.12	101.85	177.96	1	1	1CXA;A Gly 58:NH	1EQX A; Ser4 :O
7(S7)	**-10.03**	**-5.88**	**44.86**	**49.29**	**0**	**0**	**--**	**-**
8(S4)	**-9.88**	**-5.52**	**57.68**	**90.05**	**1**	**1**	**1CXA;A Gly 58:NH**	**1EQX A; Ser4 :O**
9(S8)	-8.95	-4.30	277.38	707.78	3	1	1CXA;A Gly 49:NH, 1CXA;A Tyr 57:HH 1CXA;A Trp 71:HH	1EQX A; Ser4 :O
10(S2)	**-10.9**	**-4.30**	**28.53**	**707.58**	**0**	**0**	**-**	**-**

***In-silico* ADME studies:**

An *in-silico* ADME computational study of the synthesized compounds was performed by determination of Lipinski's parameters. Calculations were performed using "Molinspiration online property calculation toolkit" and "OSIRIS property explorer".

Table 8

Lipinski properties of the synthesized compounds

Comp	Molecular weight	Log P	H bond donor	H bond acceptor	Molar refractivity	Number of criteria met
rule	***< 500***	***<5***	***<5***	***<10***	***40-130***	***At least 3***
1(S9)	354	0.98	3	6	98.99	All
2(S3)	335	1.60	2	5	97.69	All
3(S10)	336	2.03	1	5	95.6	All
4(S1)	430	5.66	1	4	128.8	4
5(S5)	342	3.79	2	4	103.3	All
6(S6)	386	2.46	3	5	110.14	All
7(S7)	367	3.73	1	5	108.12	All
8(S4)	358	3.50	3	5	104.98	All
9(S8)	421	3.52	4	7	114.73	All
10(S2)	366	4.33	1	4	110.32	All

Chapter-VII

Chapter VII

Pharmacological evaluation

Anticancer activity

Introduction

Tumor is a mass of tissues which proliferate rapidly, spread throughout the body and may eventually cause death of the host. Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacies due to the development of various side effects. This fostered our attempts to evaluate various synthetic drugs against cancer as they are less likely to cause serious side effects.

Selection Grouping and Acclimatization of Laboratory Animal

Male Swiss albino mice (20-25 gm) were produced from animal experimental laboratory, and used throughout the study. They were housed in micro nylon boxes in a control environment (temp 25±2°C) and 12 hrs dark /light cycle with standard laboratory diet and water *ad libitum.* The study was conducted after obtaining institutional animal ethical committee clearance. As per the standard practice, the mice were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Induction of cancer using DLA cells

Dalton's Lymphoma ascites (DLA) cells were supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino

mice by intraperitoneally transplantation. While transforming the tumor cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1 x 10^6, this dilution was given intraperitonealy. Let the tumor grow in the mice for minimum seven days before starting treatments.

Treatment Protocol

Swiss Albino mice were divided in to nine group of six each. All the animals in eight groups were injected with DLA cells (1 x 10^6 cells per mouse) intraperitoneally, and the remaining one group is normal control group.

Group 1 served as the normal control.

Group 2 served as the tumor control. Group 1 and 2 receives normal diet and Water.

Group 3 served as the positive control, was treated with injection fluorouracil at 20mg/kg body weight, Intraperitoneally.

Group 4 Served as a treatment control group and was administered synthetic drug (S2) in a dose of 10mg/kg intraperitoneally.

Group 5 Served as a treatment control group and was administered synthetic drug (S4) in a dose of 10mg/kg intraperitoneally.

Group 6 Served as a treatment control group and was administered synthetic drug (S5) in a dose of 10mg/kg intraperitoneally.

Group 7 Served as a treatment control group and was administered synthetic drug (S10) in a dose of 10mg/kg intraperitoneally.

Group 8 Served as a treatment control group and was administered synthetic drug (S1) in a dose of 10mg/kg intraperitoneally.

Group 9 Served as a treatment control group and was administered synthetic drug (S3) in a dose of 10mg/kg intraperitoneally.

Treatment

In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days.

On day 14, after the last dose, all mice from each group were sacrificed by euthanasia. Blood was withdrawn from each mouse by retro orbital puncture or bleeding and the following parameters were checked.

1. Hematological parameters

a. WBC count

b. RBC count

c. Hb content

d. Platelet count

e. Packed cell volume

2. Serum enzyme and lipid profile

a. Total Cholesterol (TC)

b. Triglycerides (TG)

c. Aspartate amino Transferase (AST)

d. Alanine amino Transferase (ALT)

e. Alkaline Phosphatase (ALP)

3. Derived parameter

f. Body weight

g. Life span (%)

h. Cancer Cell Count

EVALUATION OF CLINICAL PARAMETERS

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer.

$$\text{Cell count} = \frac{\text{No of cells} \quad \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Hematological parameters

i) WBC count

ii) RBC count

iii) Platelet count

iv) Hemoglobin

v) Packed Cell Volume

i) WBC count

The total WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice.

ii) RBC and Hb

RBC and Hb content decreases with tumor bearing mice when compared with Normal control mice.

iii) Platelets

In Hodgkin lymphoma, increased in platelet count often reported in laboratory finding. Hence, I investigated this parameter in the study.

iv) Packed cell volume

In any case of anemia the packed cell volume is decreases.

SERUM ENZYME AND LIPID PROFILE

The serum was analyzed for the following parameters

(a) Aspartate amino Transferase (AST)

(b) Alanine amino Transferase (ALT)

(c) Alkaline Phosphatase (ALP)

(d) Total Cholesterol (TC)

(e) Triglyceride (TG)

1. TOTAL CHOLESTEROL AND TRIGLYCERIDE (lipid profile)

Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported. Hence I investigated this parameter in the study.

2. LIVER ENZYMES (AST, ALT, ALP).

Abnormal liver function seen in patient with Hodgkin lymphoma, that these liver enzyme levels markedly increase in tumor bearing mice. ALP is an enzyme mainly derived from the liver, bones and in lesser amount from intestines, placenta, kidneys and leukocytes. An increase in ALP levels in the serum is frequently associated with the variety of disease. ALP comprises a group of enzyme that catalyzes the phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate.

Markedly elevated serum ALP, hyperalakline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis. Hence, I investigated this parameter in this study.

DERIVED PARAMETERS

1. Body weight:

All the mice were weighed, from the beginning to 15th day of the study. Average increase in body weight on the 15th day was determined.

2. Percentage increase in life span (ILS)

% ILS was calculated by the following formulae

Life span of treated group

$$\%ILS = \frac{\text{Life span of treated group}}{\text{Life span of control group}} - 1 \text{ x } 100$$

- All biochemical investigations were done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.
- Hematological test are carried out in COBAS MICROS OT 18 from Roche.
- Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample.

Table No. 9

Effect of various synthetic drugs on Hematological parameters

TREAT -MENT	Total WBC Cells /mlx10^3	Rbc Count Mill/cu mm	Hb Gm/dl	PCV %	Platelets Lakhs/cum m
G1	9.96 ±1.22	4.33±0.87	12.35 ±1.08	14.63±2.21	3.12±0.66
G2	14.32 ±2.45^{a**}	2.40±0.43^{a**}	7.09 ±0.93^{a**}	30.55±3.55^{a**}	1.54±0.44^{a**}
G3	11.26 ±1.68^{b**}	4.12±0.85^{b**}	11.0 ±1.42^{b**}	17.40±1.33^{b**}	2.63±0.68^{b**}
G4	13.76±2.10^{b*}	2.93±0.44^{b*}	9.25±0.94^{b*}	24.08±2.62^{b*}	1.93±0.37^{b*}
G5	12.72 ±1.90^{b**}	3.06±0.62^{b**}	10.25±1.20^{b**}	23.07±1.28^{b**}	2.12 ±0.62^{b**}
G6	12.50 ±1.78^{b**}	3.13±0.58^{b**}	10.34±1.32^{b**}	22.97±1.26^{b**}	2.18 ±0.28^{b**}
G7	12.36 ±1.35^{b**}	3.20±0.68^{b**}	10.40±1.40^{b**}	22.35±1.21^{b**}	2.26 ±0.45^{b**}
G8	12.05 ±1.08^{b**}	3.25±0.72^{b**}	10.55±1.55^{b**}	20.45±1.14^{b**}	2.29 ±0.65^{b**}
G9	11.70 ±1.29^{b**}	3.35±0.78^{b**}	10.68±1.62^{b**}	19.24±1.32^{b**}	2.31 ±0.58^{b**}

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Positive control, G_4 – Treatment control (S2), G5 – Treatment control (S4), G6 – Treatment control (S5), G7 – Treatment control (S10), G8 – Treatment control (S1), G9 – Treatment control (S3).

All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from control (G_1) at $P < 0.001$

*b – Values are significantly different from cancer control (G_2) at $P < 0.05$

**b – Values are significantly different from cancer control (G_2) at $P < 0.01$

Table No.10
Effect of various synthetic drugs on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	99.08±3.50	120.81±2.34	36.45 ±1.17	32.27 ±1.24	125.09 ±2.18
G_2	140.86±4.54^{a**}	206.14±4.63^{a**}	85.0±2.69^{a**}	60.18±2.55^{a**}	240.18±4.26^{a**}
G_3	110.44±3.90^{b**}	154.40±2.62^{b**}	55.22 ±1.56^{b**}	40.40±1.52^{b**}	160.26±2.23^{b**}
G_4	135.39±3.53^{b*}	185.83±2.27^{b*}	78.67 ±2.19^{b*}	56.25±1.92^{b*}	230.28±2.31^{b*}
G_5	129.29±3.42^{b**}	178.46±2.35^{b**}	75.49±2.58^{b**}	54.52 ±1.69^{b**}	221.34±2.56^{b**}
G_6	125.45±2.92^{b**}	172.58±2.44^{b**}	74.61±2.65^{b**}	51.46 ±1.54^{b**}	213.21±2.36^{b**}
G_7	123.34±2.52^{b**}	169.39±2.28^{b**}	72.42±2.46^{b**}	49.65 ±1.39^{b**}	206.19±2.54^{b**}
G_8	120.26±2.48^{b**}	164.65±2.39^{b**}	70.57±2.62^{b**}	46.39 ±1.61^{b**}	194.24±2.46^{b**}
G_9	118.48±2.62^{b**}	160.52±2.24^{b**}	69.44±2.54^{b**}	44.42 ±1.48^{b**}	185.17±2.28^{b**}

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Positive control,
G_4 – Treatment control (S2), G5 – Treatment control (S4), G6 – Treatment control (S5),
G7 – Treatment control (S10), G8 – Treatment control (S1), G9 – Treatment control (S3).
All values are expressed as mean ± SEM for 6 animals in each group.
**a – Values are significantly different from control (G_1) at $P < 0.001$
*b – Values are significantly different from cancer control (G_2) at $P < 0.05$
**b – Values are significantly different from cancer control (G_2) at $P < 0.01$

Table No.11

Effect of synthetic drug on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Cancer cell count ml X 10^6
G_1	6	>>30 days	2.12±0.44	-
G_2	6	48%	$7.64\pm0.95^{a**}$	$2.72\pm0.33^{a**}$
G_3	6	92%	$3.73\pm0.52^{b**}$	$1.25\pm0.24^{b**}$
G_4	6	66%	$6.40\pm0.82^{b*}$	$2.22\pm0.40^{b*}$
G_5	6	68%	$6.58\pm0.59^{b**}$	$2.09\pm0.39^{b**}$
G_6	6	69%	$6.25\pm0.64^{b**}$	$1.94\pm0.41^{b**}$
G_7	6	70%	$5.94\pm0.45^{b**}$	$1.82\pm0.26^{b**}$
G_8	6	71%	$5.76\pm0.38^{b**}$	$1.73\pm0.34^{b**}$
G_9	6	74%	$5.58\pm0.46^{b**}$	$1.66\pm0.28^{b**}$

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Positive control, G_4 – Treatment control (S2), G5 – Treatment control (S4), G6 – Treatment control (S5), G7 – Treatment control (S10), G8 – Treatment control (S1), G9 – Treatment control (S3).

All values are expressed as mean ± SEM for 6 animals in each group.

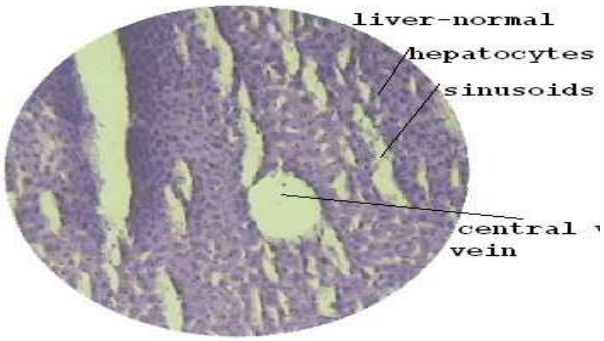
**a – Values are significantly different from control (G_1) at $P < 0.001$

*b – Values are significantly different from cancer control (G_2) at $P < 0.05$

**b – Values are significantly different from cancer control (G_2) at $P < 0.01$

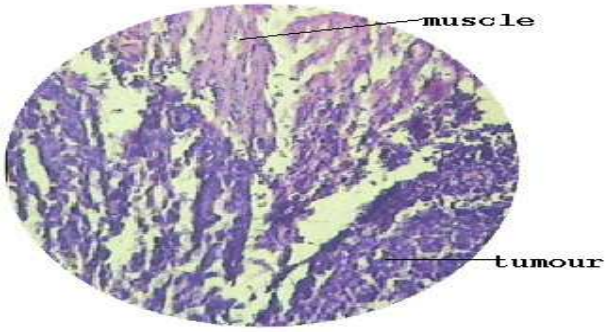
HISTOPATHOLOGICAL RESULTS

Figure No.4–NORMAL CONTROL



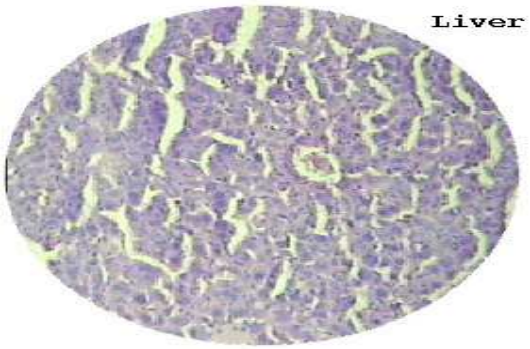
SECTION SHOW STRUCTURE OF LIVER WITH SHEETS OF HEPATOCYTES SEPARATED BY SINUSOIDS CARTIAL VEIN & PORTAL TRACT APPEAR NORMAL

Figure No.5–TUMOR CONTROL



SECTION SHOWS STRUCTURE OF LIVER presented hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, Kuffe cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate

Figure No.6–STANDARD CONTROL (5-FLUORO URACIL)

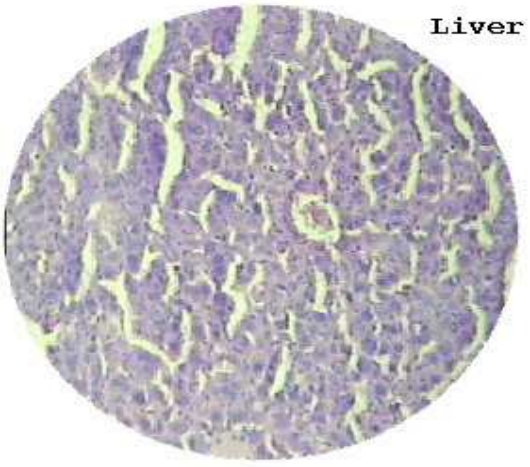


SECTION SHOW STRUCTURE OF LIVER presented mild hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Figure No.7–TREATMENT CONTROL (SYNTHETIC DRUG-S2)

SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate

Figure No.8–TREATMENT CONTROL (SYNTHETIC DRUG-S4)



SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, less Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

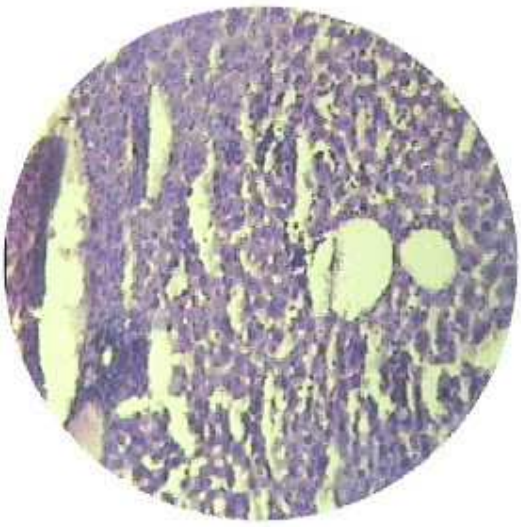
Figure No.9–TREATMENT CONTROL (SYNTHETIC DRUG-S5)

SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Figure No.10–TREATMENT CONTROL (SYNTHETIC DRUG-S10)

SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Figure No.11–TREATMENT CONTROL (SYNTHETIC DRUG-S1)



SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Figure No.12–TREATMENT CONTROL (SYNTHETIC DRUG-S3)

SECTION SHOW STRUCTURE OF LIVER presented moderate hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, No Kuffe cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate.

Figure-13

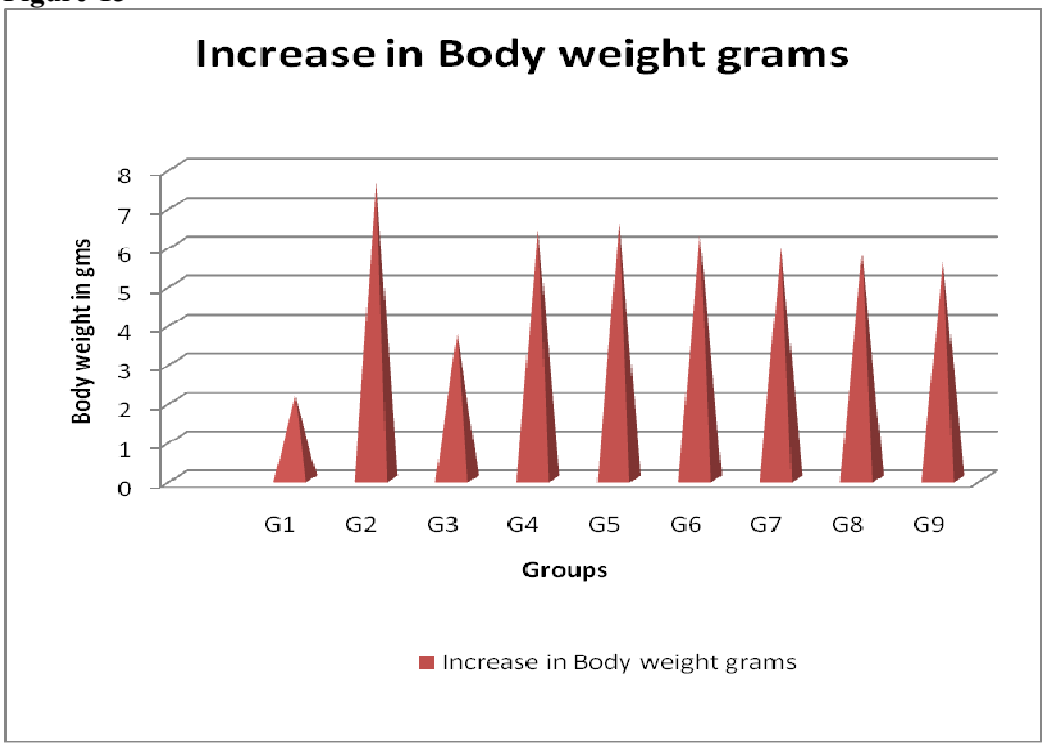


Figure-14

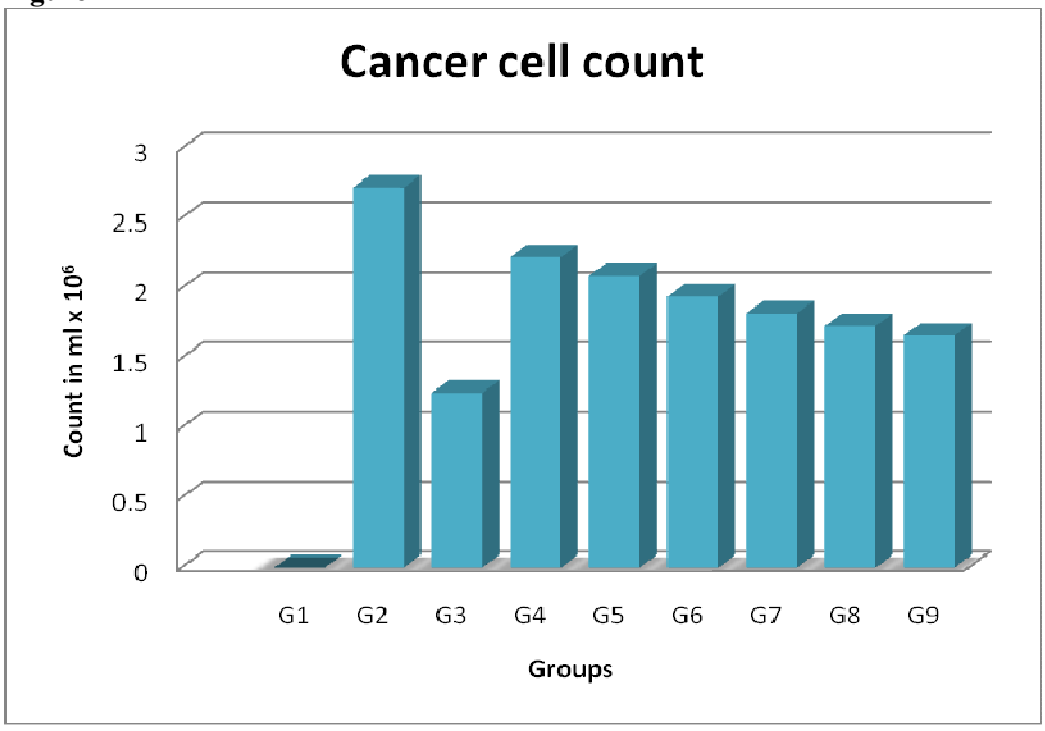


Figure-15

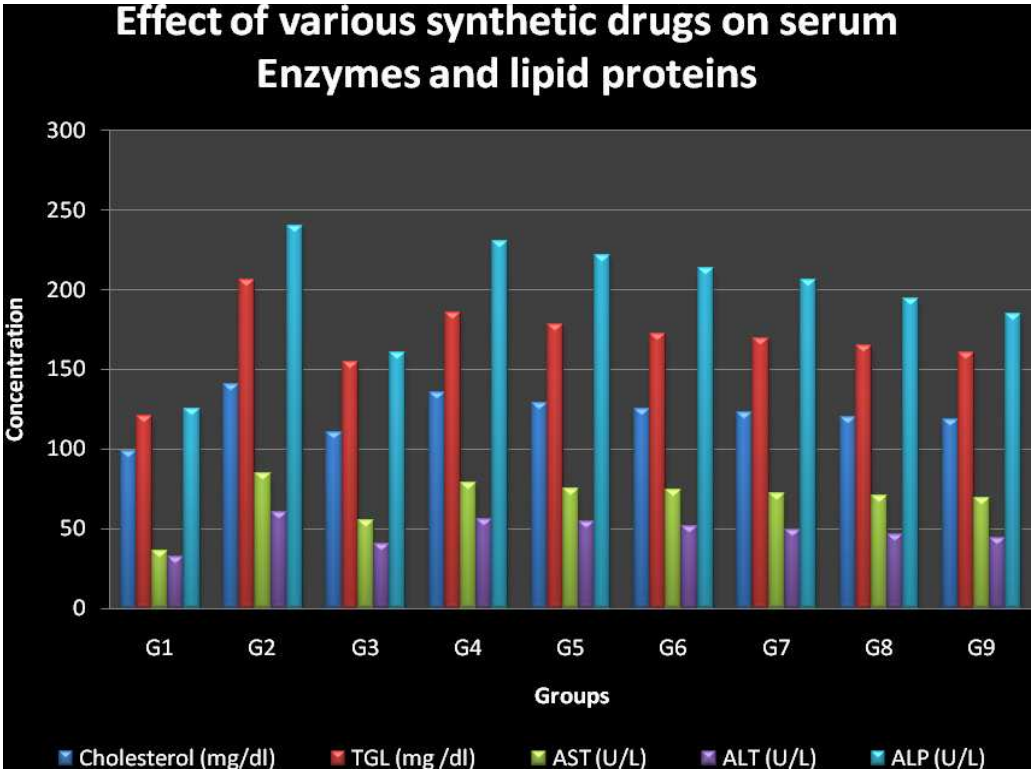
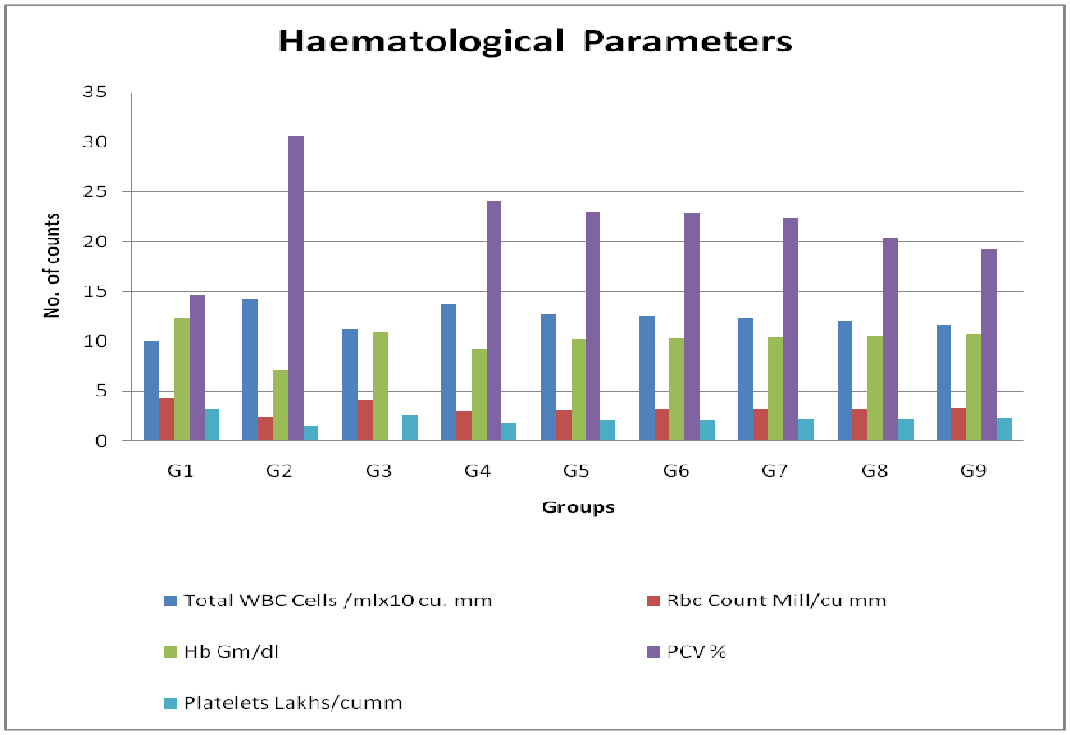


Figure-16



Anti-inflammatory activity

The anti-inflammatory activities of synthetic drugs at a dose of 10 mg/kg doses were evaluated using carrageenan-induced paw edema method. The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and edema.

REQUIREMENTS:

Animal : Albino rat (180-200 g)

Drugs and chemicals : Carrageenan (1%w/v), Diclofenac sodium (standard), Carboxy methyl cellulose (1%w/v),

Digital plethysmo meter : U G O Basile (Italy)

Test compounds : Synthetic drugs such as S2, S1, S7, S4, S5, and S6.

METHOD:

Anti-inflammatory activity was performed by the following procedure of Bhandri et al. The animals were divided into 8 groups each having six animals. A freshly prepared suspension of carrageenan (1% w/v , 0.1 ml) was injected to the planter region of left hind paw of each rat. One group was kept as control and the animals of the other groups were pretreated with Synthetic drugs such as S2, S1, S7, S4, S5, and S6. test Compounds dissolved with 0.5 ml DMSO administered through orally 30 min before the carrageenan treatment. The paw volumes of the test compounds, standard and control groups were measured at 60,240,360 minutes of carrageenan treatment with the help of Digital plethysmometer (Ugo basile, Italy).

Mean increase in paw volume was measured and the percentage of inhibition was calculated.

% Anti-inflammatory activity = (Vc-Vt / Vc) x 100

Where, ***Vt***-mean increase in paw volume in rats treated with test compounds,

Vc-mean increase in paw volume in control group of rats.

TABLE No.12

ANTI-INFLAMMATORY ACTIVITY OF SYNTHETIC DRUGS

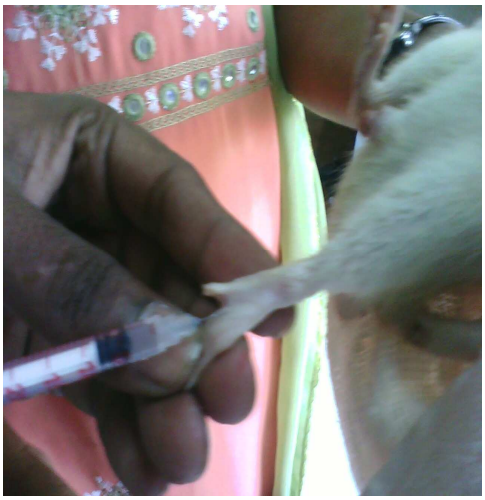
Treatment	**Dose (mg/kg)**	**Paw volume(ml) as measured by mercury displacement at 6 hour**	**Percentage inhibition of paw edema**
Group I Normal saline	10ml/kg orally	5.86±0.96	-
Group II Std	10mg/kg I.P.Diclofenac sodium	1.70±0.40	70.98%*a
Group III (S6)	10mg/kg.i.p.	2.96±0.52	49.48%*a
Group IV (S5)	10mg/kg.i.p.	2.66±0.50	54.60%*a
Group V (S4)	10mg/kg.i.p.	2.52±0.48	56.99%*a
Group VI (S7)	10mg/kg.i.p.	2.46±0.42	57.04%*a
Group VII (S1)	10mg/kg.i.p.	2.16±0.40	63.13%*a
Group VIII (S2)	10mg/kg.i.p.	2.05±0.36	65.01%*a

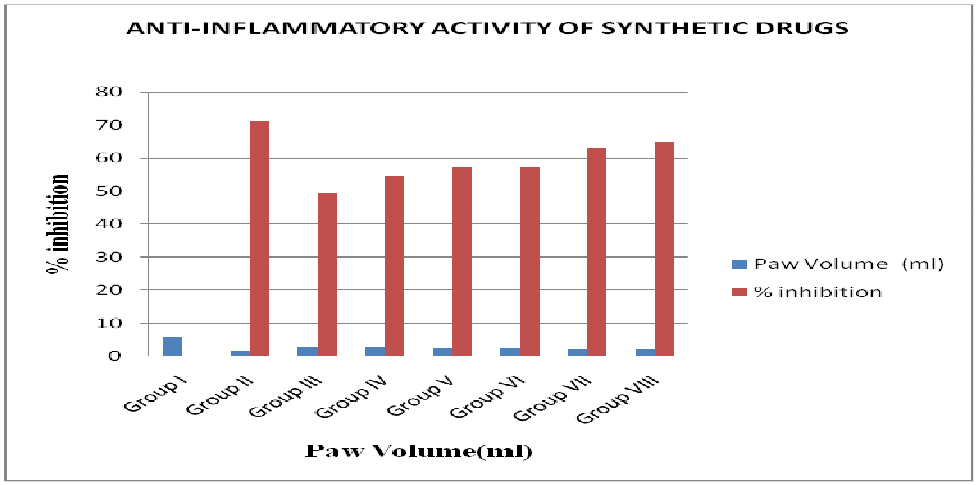
* Data are expressed as Mean ± S.E.M.

*Data were analyzed by one way ANOVA followed by Newman's keul's multiple range tests, to determine the significance of the difference between the control group and rats treated with the test compounds.

*a Values were significantly different from normal control at P< 0.01.

Figure: 17




ANTI-INFLAMMATORY ACTIVITY OF SYNTHETIC DRUGS
% inhibition
80
70
60
50
40
30
20
10
0
Group I
Group II
Group III
Group IV
Group V
Group VI
Group VII
Group VIII
Paw Volume(ml)
Paw Volume (ml)
% inhibition

Chapter-VIII

Chapter VIII

Results and Discussion

Docking studies for anti-tumor activity:

The docking results suggested that; first, the piperazinyl group (compound S3) will increase the hydrophobic binding interaction with the deep hydrophobic pocket created by Glu 121.Second, the hydrogen bonding interactions have been found, between the crucial features of compounds S10,S1,S5,S4 and S2 with the high docking scores and N–H group of ILE 141, N–H of ALA86 and Glu 121. All dock runs were conducted using Auto dock software. The 3D structure of the enzyme was used to detail intermolecular interactions between the ligand and the target protein. The prepared protein was used in the determination of the important amino acids in the predicted binding pocket.

Conclusion of molecular modeling the above molecular docking study provides useful information for understanding the structural features of CDK2 inhibitors binding mode of the newly constructed CDK2 inhibitors.

Docking studies for anti-inflammatory activity:

The binding affinities of synthesized compounds into the 3D structure of the catalytic site of COX-2 enzyme and COX-1 enzyme were determined by performing docking studies using auto dock software. Lamarckian genetic algorithm method, implemented in the program autodock 4.0.1, was employed. Binding affinity was evaluated by the binding free energies (Δ G, Kcal/mol), inhibition constant (Ki) and Hydrogen bonding (table-7). Theoretically maximum compounds showed very good

binding energy and docking energy ranging from -7.12 kJmol/1 to -10.43 kJmol/1 with COX-2.

All synthesized compounds display improved binding energies.The molecular docking studies revealed that the majority of the compounds docked in to the active sites of the receptors and exhibited H-bonding via O or –NH group. Interestingly, these compounds were having less binding energy value towards COX-1 receptor which shows that these compounds have more affinity towards COX-2 receptor. These observations together with experimental results provide a good explanation for the potent and selective inhibitory activity of **S2,S1,S7,S4,S5** and **S6.**

In vivo absorption of the new synthesized derivatives was tentatively assessed by means of theoretical calculations following Lipinski's rule of five, which establishes that the absorption or permeation of an orally administered compounds. Compounds violating more than one of these rules may present bioavailability problems. Our results (Table 8) revealed that the quinazoline derivatives presented lipophilicity less than 5, with values between 0.98 and 4.33 except compound S1 (5.66). The molecular weight of all the synthesized compounds were less than 500 (335 > MW < 430). All derivatives have number of hydrogen bond acceptors (n-ON = 4-7) and donors (n-OHNH = 1-4) in agreement with Lipinski's rule. In summary, *in silico* study pointed the quinazoline derivatives synthesized in our work as potential candidates for new anti-inflammatory and anti-tumor agents and it was found that all the ligand molecules satisfied the rule for potent inhibitors

Synthetic Methodology:

The titled compounds were synthesized in a 3 step process;

- The first step in which Anthranilic acid undergoes cyclization by the treatment with Benzoyl chloride to yield 2-phenyl 3,4-dihydro benzoxazine 4-one.
- The advantage is that it is an useful intermediate to afford 3-amino-2-phenyl quinazolin-(3H)-4one on condensation with hydrazine hydrate.
- The presence of active hydrogen in N-3 position facilates the Mannich reaction to take place with several amines to yield 3-substituted amino-2-phenyl quinazolin-(3H)-4-one derivatives.

Characterization:

- The melting points were found in an open end capillary tube method by electrically heating melting point apparatus and are uncorrected.
- The purity of the compounds were analyzed by Thin Layer Chromatography using silica gel (0.5 mm thickness) as stationary phase, employing.

  Methanol : Chloroform : Water (9:1:1) as mobile phase, spots were visualized using Iodine vapours.

The R_f value of the synthesized compounds were calculated

The characterization of the titled compounds including infrared and Nuclear magnetic resonance spectral datas & mass spectral datas were in correlation with the expected structure.

Anti-tumor activity

Effect on Tumor Growth

In the DLA tumor control group, the average life span of animal was found to be 48% where as synthetic drug such as S2, S4, S5, S10, S1 and S3 at dose of 10mg/kg body weight increase the life span to 66%, 68%, 69%, 70%, 71%, and 72%

respectively. These values were significant. However the average life span of 5- FU treatment was found to be 92%, indicating its potent antitumor nature.

It was also supported by the significant reduction in packed cell volume and viable Tumor cell count in various synthetic compounds at dose of 10 mg/kg treatment when compared to the DLA tumor control. (Table No .11).

Effect on Hematological Parameters

As shown in (Table No.9) RBC, Hgb, Platelets were decreased and WBC count was significantly increased in the DLA control group compared to the normal control group. Treatment with various synthetic compounds such S2, S4, S5, S10, S1 and S3 as at dose of 10mg/kg body weight significantly increases the Hgb content, RBC, Platelets and significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the synthetic drugs. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better result in all these parameters.

Effect on Biochemical Parameters

The inoculation of DLA cells caused significantly increase in the level of Total Cholesterol, Aspartate amino Transferase, Alanine amino Transferase, Alkaline Phosphatase in the tumor control animals(G_2), when compared to the normal group. The treatment with various synthetic compounds such as at dose of 10mg/kg body S2, S4, S5, S10, S1 and S3 weight reversed these changes towards the normal level. (Table No. 10) All the value was found to be significant. The treatment with standard 5- FU also gave similar results.

In DLA tumor bearing, a regular rapid increase in Ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in Ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. Treatment with various synthetic compounds such as at dose of 10mg/kg body weight S2, S4, S5, S10, S1 and S3 inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals. It may be concluded that various synthetic compounds such as S2, S4, S5, S10, S1 and S3 by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DLA bearing mice. Thus various synthetic compounds such as S2, S4, S5, S10, S1 and S3 have antitumor activity against DLA bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelo suppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with various synthetic compounds such as S2, S4, S5, S10, S1 and S3 brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels significantly. This clearly indicates that various synthetic compounds such as S2, S4, S5, S10, S1 and S3 possess protective action on the haemopoietic system.

It was reported that the presence of tumor in the human body or in the experimental animals is known to affect may function of the liver. The significantly elevated level of total cholesterol, TG, AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The

significant reversal of these changes towards the normal by various synthetic drugs treatments.

In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with various synthetic compounds such as S2, S4, S5, S10, S1 and S3 supported the potent antitumor effect of the drugs.

Anti- inflammatory activity

Various synthetic drugs such as S2, S1, S7, S4, S5, and S6 at a dose of 10mg/kg were tested for their Anti- inflammatory activity by using carrageenan Induced rat paw edema method and the results are tabulated in table no 12. The results reveals that synthetic drugs S2, S1, S7, S4, S5, and S6 at 10mg/kg doses possesses significant Anti- inflammatory activity when compared to control group at $p<0.01$.

Conclusion

- Novel 2-phenyl 3-substituted amino quinazolin-4(3H)ones were synthesized by a 3 step process using Anthranilic acid and Benzoyl chloride as starting materials.
- The melting points were found for the synthesized compounds and are uncorrected. The purity of the synthesized compounds were analyzed by Thin Layer Chromatography method.
- The structures of the synthesized compounds has been elucidated by Infra-red, Nuclear magnetic resonance spectroscopy & Mass spectroscopy.
- The anti-cancer activity of the synthesized compounds (10mg/kg) were screened by using Dalton's Lymphoma Ascites (DLA) cells in albino mice against the standard drug 5-FU (20mg/kg).
- The results obtained showed that the attachments of piperazine, Dicyclohexylamine,Morpholine,aniline,4-aminophenol,Indole moieties to the quinazolin-4(3H)-one ring exhibited significant anti-cancer activity.
- The anti-inflammatory activity of the synthesized compounds (10mg/kg) were screened by carrageenan induced paw edema method in *albino rats* against the standard drug Diclofenac sodium (10mg/kg).
- From the obtained results quinazolin-4(3H)-one ring having substitutions like Indole, Dicyclohexylamine, Benzimidazole, 4-aminophenol, aniline, PABA showed significant anti-inflammatory activity.
- Future investigations can be made to study its pharmacokinetic parameters, potency, efficacy, drug interactions, side effects of the titled compounds in

order to bring out the novel quinazolin-4(3H)-one derivatives as a successful drug molecules.

- Extended studies can be made to broaden the therapeutic utility of the synthesized compounds such as anti-microbial, anti-malarial, anti-tuberculosis, anti-HIV, anti-parkinsonism, anti-histaminic, local anaesthetic, anti-hypertensive and anti-viral activities.

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